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**KEYWORDS:** Pattern Reversal Evoked Potential (PREP); Electrotrigeminogram (ETG); Olfactory/Trigeminal Evoked Potential (EP); Cigarette Smoking; Nicotine; Feeling Factors; Central Nervous System (CNS); Trigeminal Nerve; Limonene; $CO_2$; Methyl Salicylate; Vanillin; Menthol

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## SUMMARY

Experiments were conducted employing pattern reversal evoked potentials (PREPs), evoked potentials to olfactory and trigeminal stimulants (EPs) and electrotrigeminograms (ETGs).

Three PREP studies were completed and a fourth is in progress. The goal of each of these studies has been to systematically explore the effects of cigarette smoking on central nervous system activity. In the Return to Baseline study, it was demonstrated that smoking produced a statistically significant decrease in PREP $P_{100}$ latencies which endured for 10 min. post-smoking. However, $P_{100}$ latencies did not return to pre-smoking baseline levels until 60 min. post-smoking. A second study examined the relationship between cigarette smoking and $P_{100}$ latencies over a four hour period. It was demonstrated that when subjects are allowed free access to cigarettes, $P_{100}$ latencies are maintained below pre-smoking values. The third study investigated the effects of cigarette smoking on $P_{100}$ latencies on a puff-by-puff basis. It was ascertained that maximal central nervous system effects are obtained while smoking, and that the effects begin to dissipate by the time the cigarette is extinguished. In the Dose/Response study the effects of a range of nicotine deliveries (0.17 - 1.65 mg/cigarette) on $P_{100}$ latencies are being examined.

Research on EPs was initiated in several studies. In a flow rate experiment, it was determined that a flow rate of 134 ml/sec produced reliable responses to all stimuli under study. A series of experiments was carried out on EPs to limonene, methyl salicylate and combinations of these odorants with $CO_2$. It was demonstrated that the stimuli were differentiable in terms of latency, amplitude and waveform morphology. In a series of concentration/response studies (in progress), the manners in which EPs change with different stimulus concentrations are being investigated. These studies appear to


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indicate that EP waveform characteristics accurately reflect stimulus intensity.

Outside EP studies performed by G. Kobal in Erlangen, W. Germany, were discussed. Kobal recorded EPs to vanillin, $CO_2$, menthol and the combination of vanillin and $CO_2$. The results of Kobal's experiments were found to basically agree with experiments performed at R&D.

Initial studies were carried out on the ETG. ETGs to high concentrations of limonene, methyl salicylate and $CO_2$ were recorded.

Plans for future PREP, EP and ETG studies were discussed.

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## I. DEFINITIONS AND ABBREVIATIONS

Amplitude-Evoked Potential - The height of some component of the multiphasic evoked potential waveform, measured either from a zero baseline, or with respect to another component.

Central Nervous System (CNS) - The brain and spinal cord.

Component/Peak-Evoked Potential - One wave of the multiphasic evoked potential waveform. It is usually measured in terms of polarity (P or N) and either its normal latency (e.g., $P_{100}$) or its ordinal relationships to other peaks (e.g., $P_1$).

Electroencephalogram (EEG) - A record of the ongoing electrical activity of the brain. It is usually recorded from the scalp via gold or silver-silver chloride electrodes positioned over the brain areas under study.

Electrotrigeminogram (ETG) - A record reflecting the hypothesized depolarization potential of the free nerve endings of the trigeminal nerve (cranial nerve V). In this case, the ETG refers to the negative potential recorded from the inner lateral region (regio respiratoria) of the human nose.

EP - In the context of this report, EP will be used to refer to the electrical response of the brain to olfactory and trigeminal stimulants.

Evoked Potential - The electrical response of the brain to the onset (usually) of a sensory stimulus. In general, evoked potentials are small multiphasic waveforms which must be signal averaged to extract them from the ongoing (and much larger) EEG.

Feeling Factors - In Cigarette Smoke - The qualities of cigarette smoke that can be classified as spicy, peppery, cool, warm, pungent, etc. Feeling factors are commonly thought to be mediated by stimulation of the trigeminal nerve in both the nasal and oral cavities.

Latency-Evoked Potential - The time from stimulus onset to the occurrence of some component (peak) of the evoked potential waveform.

msec - milliseconds

μv - microvolts

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Olfactory Evoked Potential (OEP) - A somewhat inaccurate, but generally used term to describe the cortically recorded response to stimulation of the sense of smell. The sense of smell includes olfactory (stimulation of olfactory receptors) and trigeminal (stimulation of free trigeminal nerve endings in the respiratory mucosa) components. In other words, OEPs reflect odor and/or feeling factor stimulation. In this report, the term EP will be used in place of OEP.

Pattern Reversal Evoked Potential (PREP) - The cortically recorded response of the visual system to a shifting pattern (usually a checkerboard). The PREP is the most stable and reproducible electrocortical response yet recordable in humans.

$P_{100}$, $P_1$-PREP - The primary (largest) positive component of the PREP. Its typical latency is 100.0 ± 4.0 msec. The latency of the $P_{100}$ exhibits systematic changes in response to the smoking of cigarettes of differing nicotine deliveries.

Psychophysical Data - Data which relate psychological stimulus scales to physical stimulus scales (e.g., perceived loudness to sound wave amplitude).

Signal Averaging - Evoked Potential - The process by which an evoked potential is extracted from the ongoing EEG. Since the evoked potential is time-locked to stimulus presentation, it can be computer-summated. The ongoing EEG (and artifacts) is random with respect to stimulus presentation and is averaged to zero. The number of stimulus presentations necessary to record a clear evoked potential varies with the signal (evoked potential) to noise (EEG and artifacts) ratio.

Trigeminal Nerve - The fifth cranial nerve, widely distributed throughout the head. Intense stimulation of the trigeminal nerve is associated with the sensation of pain and reflexes such as tearing, salivation and inhibition of respiration. Modest trigeminal stimulation may be pleasurable and is associated with feeling factors such as spiciness, pungency, cooling, warmth, etc.

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## II. OBJECTIVES

The overall objectives of Project 1620 (Electrophysiological Studies) are to: (1) gain further understanding of the electrophysiological consequences of cigarette smoking, with a particular emphasis on nicotine; and (2) develop and utilize new techniques which will permit the quantification of physiological responses to smoke constituents and tobacco flavorants. Specific objectives are:

I. Pattern Reversal Evoked Potential (PREP) Studies

A. To utilize the PREP to study:

1. the relationship between cigarette smoking and central nervous system activity.

2. the role of nicotine in smoking behavior.

II. Evoked Potentials to Olfactory and Trigeminal Stimulants (EPs)

A. To develop an EP program to study:

1. central nervous system responses to current and new or alternative tobacco flavorants.

2. feeling factors in tobacco flavorants and smoke constituents.

III. Electrotrigeminograms (ETGs)

A. To develop an ETG program to:

1. gain direct knowledge about irritative factors in whole smoke and smoke constituents.

2. gain additional insight on the role of the trigeminal nerve with respect to feeling factors in tobacco flavorants and smoke constituents.

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## III. Inter-Individual Differences in Evoked Potential Research

Large inter-individual differences are found in all electrophysiological studies on humans. No studies of which the authors are aware have failed to find a statistically significant difference among individuals, irrespective of possible treatment effects.

Given this fact, differences among individuals must be treated statistically. The design of choice in most experiments is a repeated measures analysis of variance.[1] In the design, the error variability attributable to individual differences is eliminated. In other words, subjects are handled as a treatment effect, and as a consequence, the variability associated with inter-individual differences is isolated. Once isolated, the effects due to the experimental treatments can be dealt with without the inflated variability due to differences among subjects.

A simple example might serve to illustrate how a repeated measures analysis of variance allows for the analysis of experimental treatments irrespective of inter-individual differences.

Table 1

| | LOW | MEDIUM | HIGH |
|---|---|---|---|
| $S_1$ | 4 | 8 | 10 |
| $S_2$ | 20 | 25 | 30 |
| $S_3$ | 30 | 32 | 38 |
| $S_4$ | 6 | 9 | 12 |
| $S_5$ | 10 | 14 | 16 |
| $\bar{X}$ = | 14.0 | 17.6 | 21.2 |

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In Table 1 are the hypothetical results of an evoked potential experiment performed on five subjects ($S_1$-$S_5$). The subjects were tested using three stimulus concentrations (low, medium and high).

Large differences among subjects are apparent, irrespective of treatment. Yet, if those differences are isolated in the analysis, a statistically significant treatment effect would be obtained and it would be seen that scores tend to increase with increasing stimulus concentrations.

The statistical logic and assumptions of the repeated measures analysis of variance can be found in most books on experimental design. Here, it is sufficient to note that the analysis is very sensitive and statistically robust.[1]

All of the evoked potential experiments performed at R&D employ experimental designs which statistically eliminate inter-individual differences. Hence, it has been possible to detect differences among experimental treatments, even where large differences among subjects have been encountered.

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## IV. PATTERN REVERSAL EVOKED POTENTIAL (PREP) STUDIES

### A. General Considerations

The usefulness of the PREP for assessing the CNS responses to cigarette smoking has been demonstrated in a number of studies.[2,3,4] In particular, it has been shown that when other variables are controlled the effects that are obtained on the PREP appear to be attributable to nicotine. Specifically, cigarette smoking produces a decrease in the latency of the primary positive component ($P_{100}$) of the PREP. The magnitude of this effect is related to nicotine delivery in a dose-dependent fashion. The $P_{100}$ latency decrease appears to be related to the stimulating effects of nicotine on the visual system,[5] and it may reflect a post-smoking facilitation of visual information processing.[6]

The effects of cigarette smoking on the PREP are extremely reliable, and the findings with respect to the $P_{100}$ have been replicated in numerous studies over the last three years.[2,3,4] Therefore, it is now possible to conduct highly specific and systematic studies on the nature of the events that occur in the brain as a consequence of cigarette smoking.

In this report, four parametric experiments on the PREP and cigarette smoking are described. In the first experiment, the issue of how long CNS effects endure following the smoking of a single cigarette is addressed (Return to Baseline study). The second experiment investigates the nature of CNS effects produced by repeated smoking (Smoking Over a Four Hour Period study). The third study involves a puff-by-puff analysis of smoking-produced CNS effects (Puff-by-Puff Analysis of Cigarette Smoking study). Finally, a study on the dose/response relationships between nicotine delivery and CNS effects is described (Dose/Response study - in progress).

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B. Return to Baseline Study

How long do CNS effects endure following the smoking of a single cigarette? The answer to this question might help to illuminate the timing mechanisms which regulate the rate at which cigarettes are smoked. It has been postulated that both cigarette smoking and smoking rate are, at least in part, determined by CNS effects.[7] Therefore, it should be possible to address this question by determining the time course over which PREP $P_{100}$ latency effects are observed following smoking.

Previous work[3] indicated that the PREP $P_{100}$ latency decrease produced by smoking endured at least 20 min. Although the latency effect was statistically significant for only 5 min. post-smoking, it was obvious from an inspection of the data that, during the 20 min. post-smoking interval employed in this study, the CNS had not returned to its pre-smoking state.

Preliminary research indicated that residual effects of cigarette smoking on $P_{100}$ latency were still visible for at least 60 min. post-smoking. Hence, in this study, it was decided that it would be necessary to examine the effects of smoking from 0 to 70 min. post-smoking.

The subjects for this experiment were 10 R&D employees who had been over-night smoke deprived. The technique for recording the PREP has been described elsewhere.[3] At the beginning of each test session pre-smoking baselines were obtained. The subjects were then required to smoke a cigarette that delivered 0.94 mg nicotine in mainstream smoke using a controlled smoking procedure.[2] Post-smoking PREPs were then recorded at 0, 10, 20, 30, 40, 50, 60 and 70 min.

The results are shown in Figure 1. A multiple range test revealed that cigarette smoking resulted in a decrease in $P_{100}$ latency which was statistically significant at 0 (3.19 msec) and 10 (1.85 msec) min. post-smoking. By

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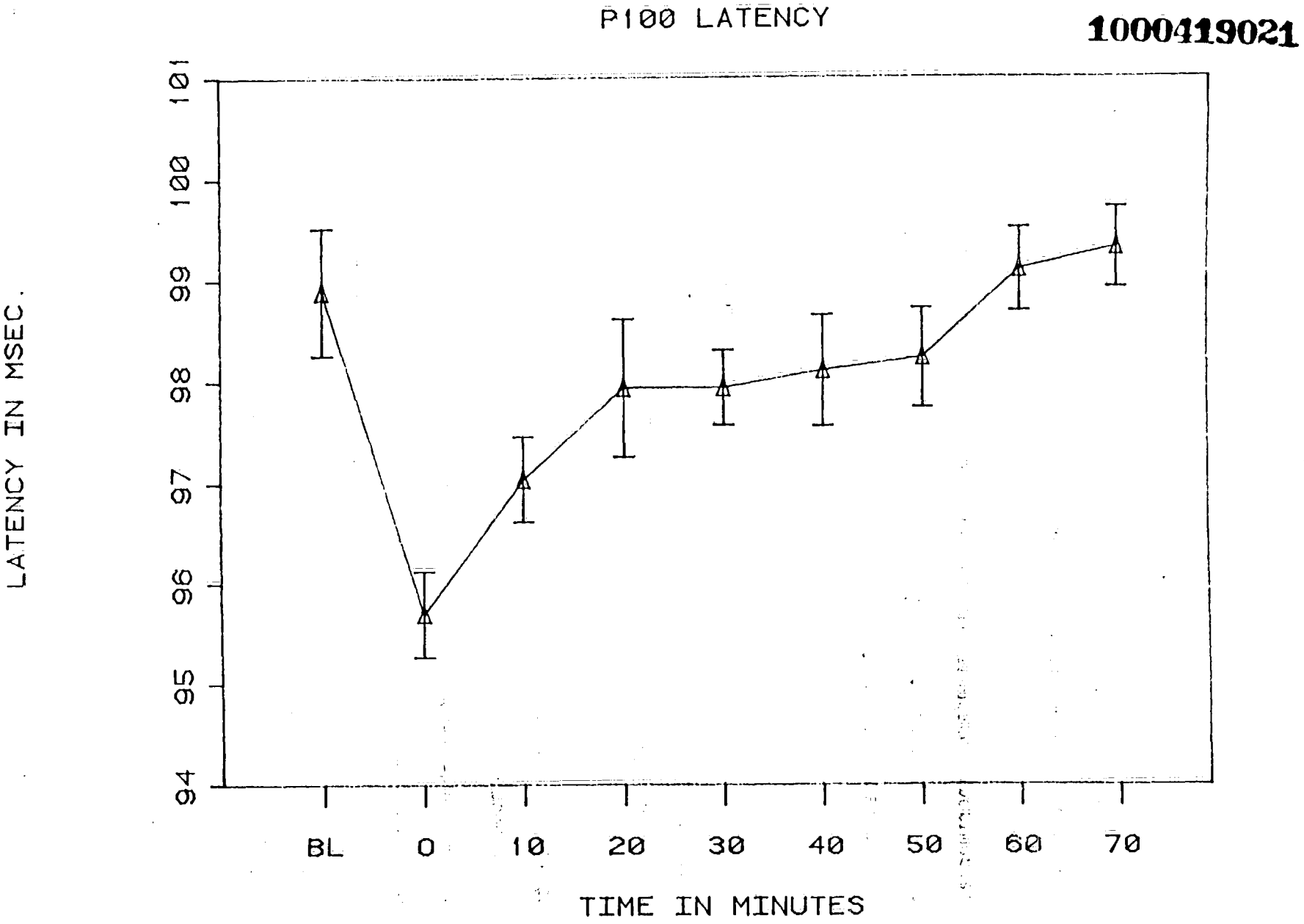
Figure 1.




The effects of cigarette smoking on $P_{100}$ latency. The latency reduction is statistically significant at 0 and 10 min. post smoking. Vertical lines through data points represent standard error values. (n = 10 PREPs at each data point.)



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20 min. post-smoking, the latency decrease was no longer statistically different from pre-smoking baseline values.

Although no longer significant, the $P_{100}$ latency did not attain its pre-smoking baseline value until between 50 and 60 min. after smoking. These findings suggest that the effects of smoking a single cigarette on the $P_{100}$ latency are rather short-lived, being maximal immediately post-smoking, and by 1h post-smoking the effects have entirely dissipated.

Since most moderate smokers smoke at a rate of greater than one cigarette per hour, it is at least theoretically possible that they are, in part, responding to CNS cues. That is, they may be prompted to smoke their next cigarette when the CNS effects of the previous cigarette have begun to wane.

In order to assess the validity of the hypothesis set forth above, it would be necessary to examine the CNS events occurring just prior to smoking. This problem was addressed in the next study.

C. Smoking Over a Four Hour Period

The majority of the electrophysiological research conducted at R&D has been directed toward examining the relatively short-term effects of cigarette smoking on CNS activity. Specifically, the standard experimental protocol has been to assess the acute effects of smoking a single cigarette. Consequently, it has not been possible to determine whether the effects that are observed in the acute situation apply to normal conditions, where individuals smoke repeatedly over the course of the day.

This PREP study was directed toward a more long-term examination of smoking as it occurs during a period of several hours. The conditions of the experiment were semi-naturalistic, in that the subjects were free to smoke when

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and as often as they wished. The questions of interest were: 1) whether the subjects, as a group, would maintain PREP $P_{100}$ latency values below pre-smoking baselines, and 2) for a given individual, would a return to pre-smoking $P_{100}$ latency values be observed just prior to when the subject smoked his next cigarette?

The same 10 R&D employees that served as subjects in the Return to Baseline study were used in this experiment. Once again, they were over-night smoke deprived. The PREP recording technique has been described elsewhere.[3] Baseline PREPs were obtained prior to smoking. Immediately after obtaining baselines, the subjects smoked a cigarette that delivered 0.94 mg nicotine in mainstream smoke, using a controlled smoking procedure,[2] after which a second PREP was immediately recorded. PREPs were then obtained every 10 min., or immediately after the subject smoked a cigarette. Subjects were allowed to smoke at any time during the experiment, but were required to smoke using the controlled smoking procedure.

On a separate occasion, a control recording session was obtained. In the control sessions, the subjects were not allowed to smoke. Instead, PREPs were obtained at 10 min. intervals until 20 PREPs had been recorded.

The results of an experiment on one subject are illustrated in Figure 2. In the figure, a relationship between smoking rate and $P_{100}$ latency is apparent. In general, there was a return toward pre-smoking baseline values in the PREP, recorded just prior to smoking (arrows). Similar relationships were apparent, to a lesser degree, in eight of the nine remaining subjects. In the tenth subject, no trends were observable, since the subject smoked almost continuously throughout the recording session.

When the group data for all ten subjects are plotted (Figures 3 and 4), it can be seen that $P_{100}$ latencies are very different in the smoking and



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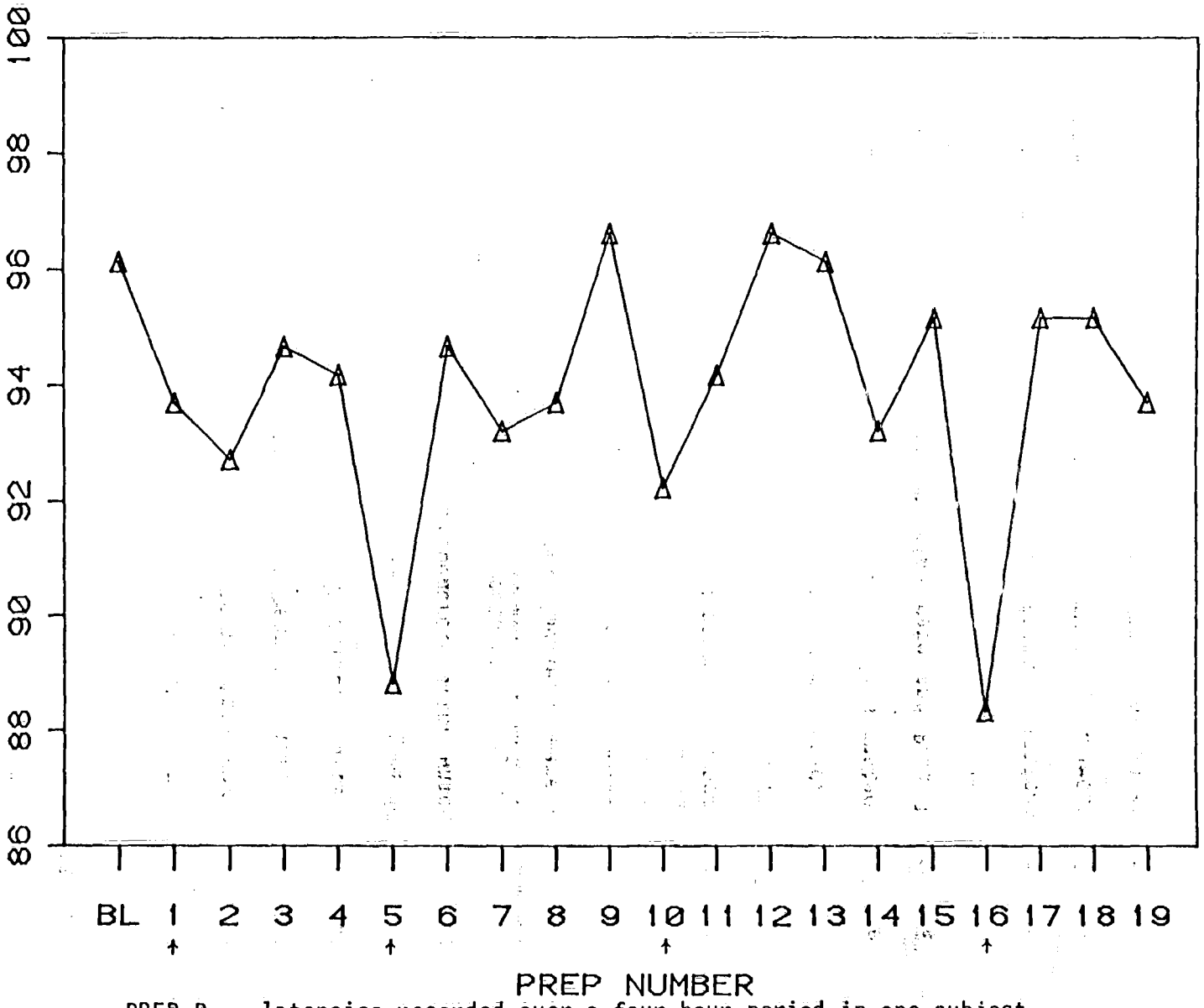
Figure 2.

SMOKING OVER 4 HOURS - SINGLE SUBJECT




PREP $P_{100}$ latencies recorded over a four hour period in one subject.
Arrow indicates recording immediately subsequent to smoking.



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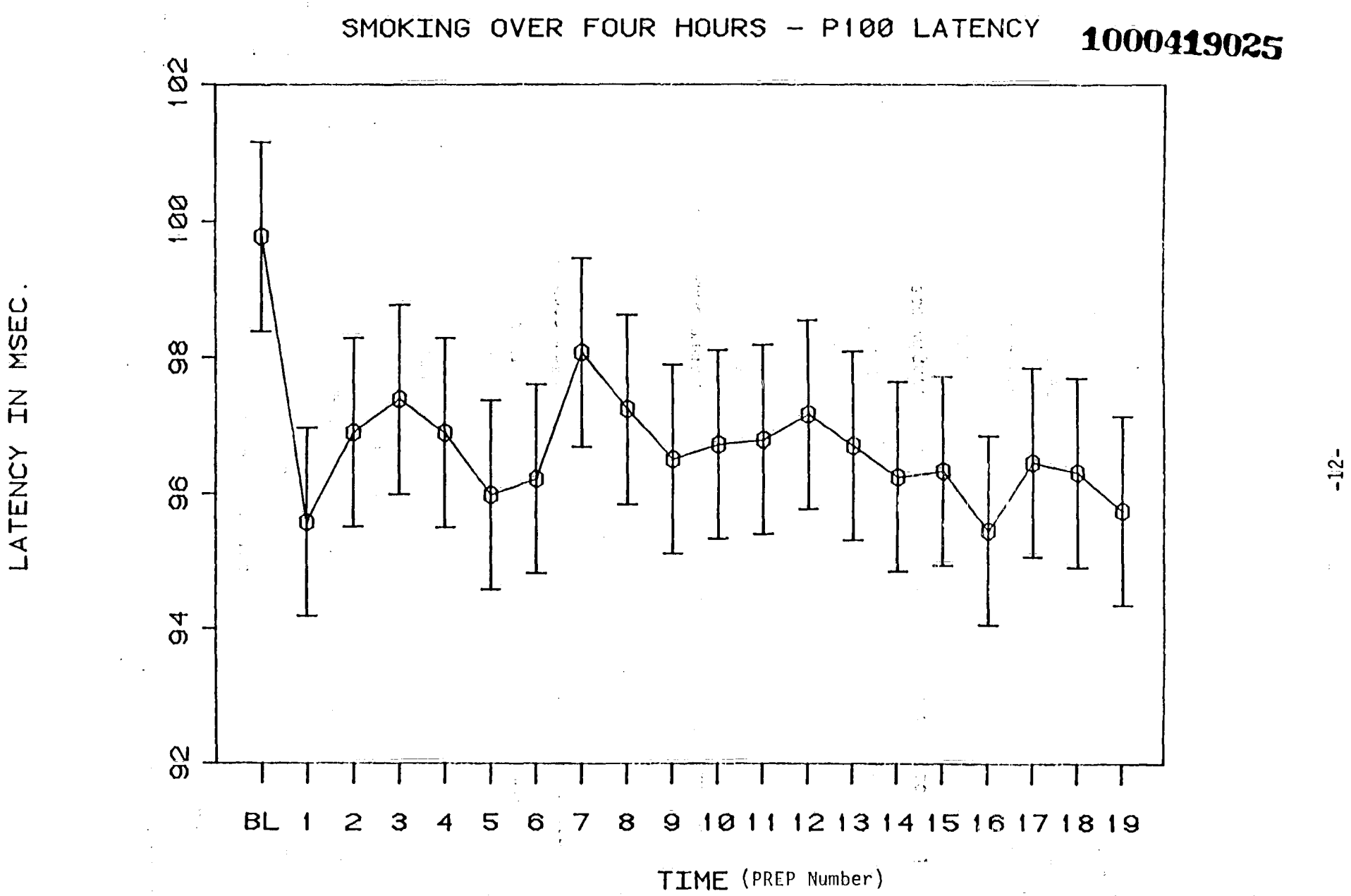
Figure 3




The effects of smoking on $P_{100}$ latency. Post-smoking reduction in latency is statistically significant for all data points except No. 7. Vertical lines through data points represent 95% confidence interval. (n = 10 PREPs per data point.)



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the non-smoking (control) condition. In the smoking condition (Figure 3), $P_{100}$ latencies remained below pre-smoking baseline values throughout the 4h recording session. In the control condition (Figure 4), $P_{100}$ latencies remained close to pre-smoking baselines. Between conditions, the overall differences in $P_{100}$ latency values were statistically significant ($p < .04$).

Multiple range tests were conducted in order to determine, within a condition, which data points were statistically different from baseline values. In the smoking condition, every data point except 7 was found to be statistically different from baseline. During the interval in which data point 7 was obtained, only one of the ten subjects smoked a cigarette. Therefore, the lack of statistical significance for this point is easily understandable. In the control condition, no data point was found to be statistically different from baseline.

Two interesting points emerge from an inspection of the data. First, there appears to be some relationship between the $P_{100}$ latency and when the next cigarette is smoked. That is, there is a tendency for $P_{100}$ latency values to progress toward pre-smoking baselines in the recordings just prior to when the subjects smoke their next cigarette. This would suggest that the $P_{100}$ latency might be a useful index for determining smoking rates. These data also suggest that smoking rate might, in part, be governed by CNS effects. It is possible that when the CNS effects of the previous cigarette have dissipated to a certain degree, the subject will desire another smoke.

Second, when the subjects were allowed to freely smoke, $P_{100}$ latency values remained below pre-smoking baseline values. These data indicate that although the effects of smoking a single cigarette are short-lived, they are maintained by repeated smoking.

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Figure 4.

NO SMOKING OVER FOUR HOURS - P100 LATENCY

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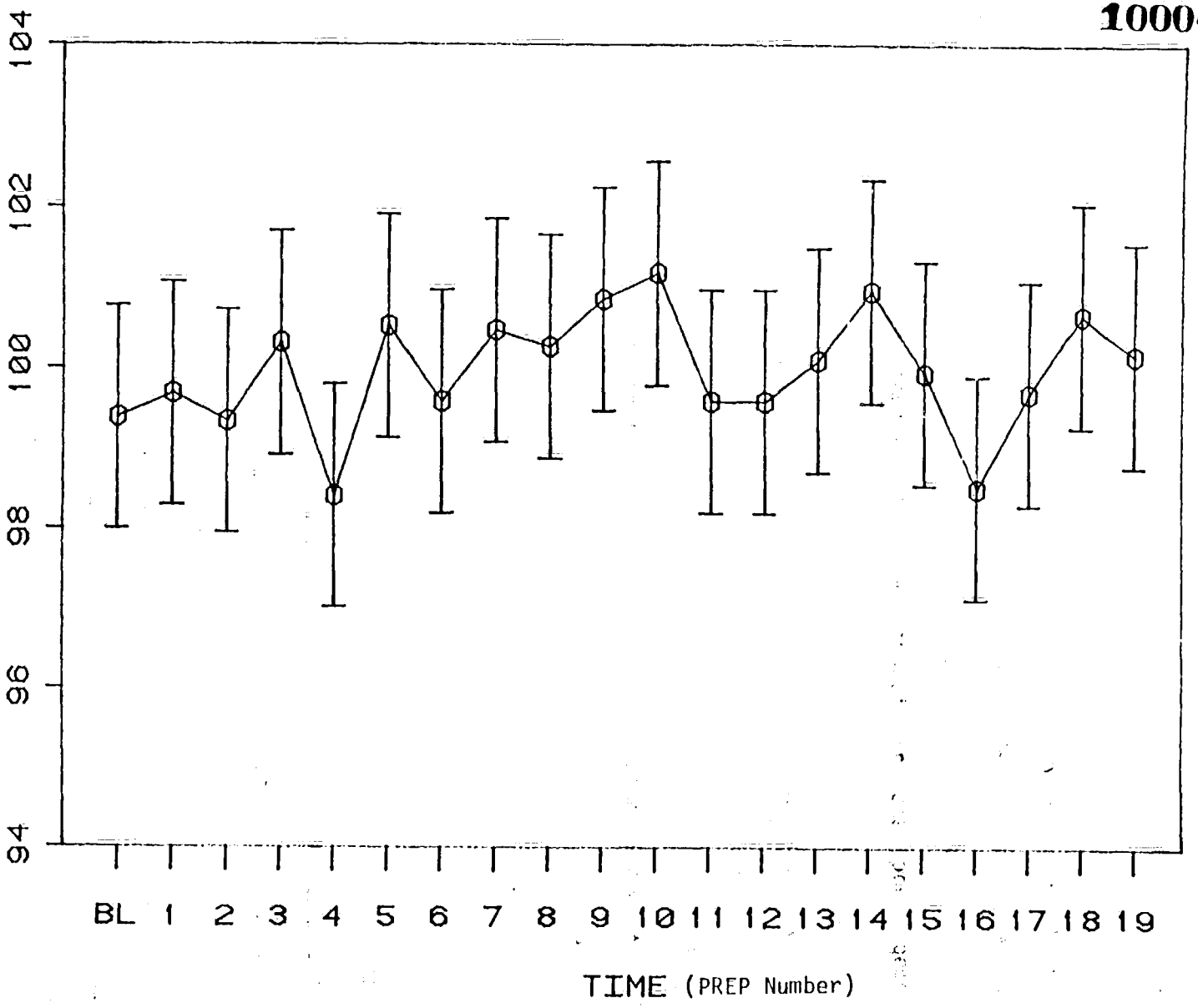


No smoking control condition. No latencies are statistically different from baseline. Vertical lines through data points represent 95% confidence interval. (n = 10 PREPs per data point.)



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As a group, the subjects did not wait until the $P_{100}$ latency returned to baseline before smoking the next cigarette. On the average, a cigarette was smoked every 30.8 min. From the Return to Baseline study (Figure 1), it can be seen that a rate of one cigarette every 30.8 min. is correlated with the flattened portion of the $P_{100}$ latency curve. Interestingly, this smoking rate is also correlated with the biological half-life of nicotine in humans.[7]

Additional study would be necessary to establish the validity of these conclusions. Specifically, it would be necessary to find a method by which CNS activity could be continuously monitored. It would then be possible to precisely determine the state of the CNS immediately prior to the initiation of smoking. This matter is currently under study.

D. Puff-by-Puff Analysis of Cigarette Smoking

The standard procedure for determining the effects of cigarette smoking on the PREP has been to record baseline latency and amplitude values, have the subject smoke a cigarette, then to record post-smoking values.[2,3] This procedure results in an overall determination of smoking effects, as measured from the time it takes to extinguish the cigarette and to record post-smoking values.

Employing the procedure described above, it has been demonstrated,[2,3] for example, that high nicotine delivery (approximately 1.0 - 1.3 mg) cigarettes produce an average PREP $P_{100}$ latency decrease of 3.0 to 3.2 msec. Lower nicotine delivery cigarettes produce lesser effects in a dose-related manner.

This study sought to determine the CNS effects which might be occurring as the subjects smoke. Specifically, the goal of the study was to conduct

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a puff-by-puff analysis of PREP $P_{100}$ latency effects. By conducting such a study, it might be possible to determine: 1) the point at which $P_{100}$ latency effects are first detectable; 2) the point at which maximal $P_{100}$ latency effects occur, and 3) a puff-by-puff profile of the manner in which the CNS is modified by cigarette smoking.

The same 10 R&D employees that served as subjects in the previous two studies were used in this experiment. Once again, they were overnight smoke deprived. The PREP recording technique has been described elsewhere.[3] Prior to smoking, baseline PREPs were obtained to 30 averaged pattern reversals. The subjects were then required to smoke a cigarette that delivered 0.94 mg nicotine in mainstream smoke, using a controlled smoking procedure.[2] Subsequent to each exhalation, PREPs were again obtained to 30 averaged reversals. The inter-puff-interval was held constant at 30 sec. This procedure was followed until a total of 8 puffs had been taken.

The entire procedure was repeated a second time, beginning approximately 10 min. after the first cigarette was smoked. Hence, two series of PREPs were obtained for each subject: one under an overnight smoke deprivation condition, and another when the subjects were not smoke deprived.

In the overnight smoke deprivation condition, a statistically significant reduction in $P_{100}$ latency was obtained after the first puff on the cigarette. The reduction in latency remained statistically different from pre-smoking baselines for the remaining seven puffs. The difference in latency values among puffs, however, was not statistically significant (see Figure 5).

It is particularly interesting to note that a $P_{100}$ latency shift of over 3.0 msec was obtained after the first puff. The magnitude of this effect is comparable to the effects obtained with a high delivery (i.e., 1.0 - 1.3 mg

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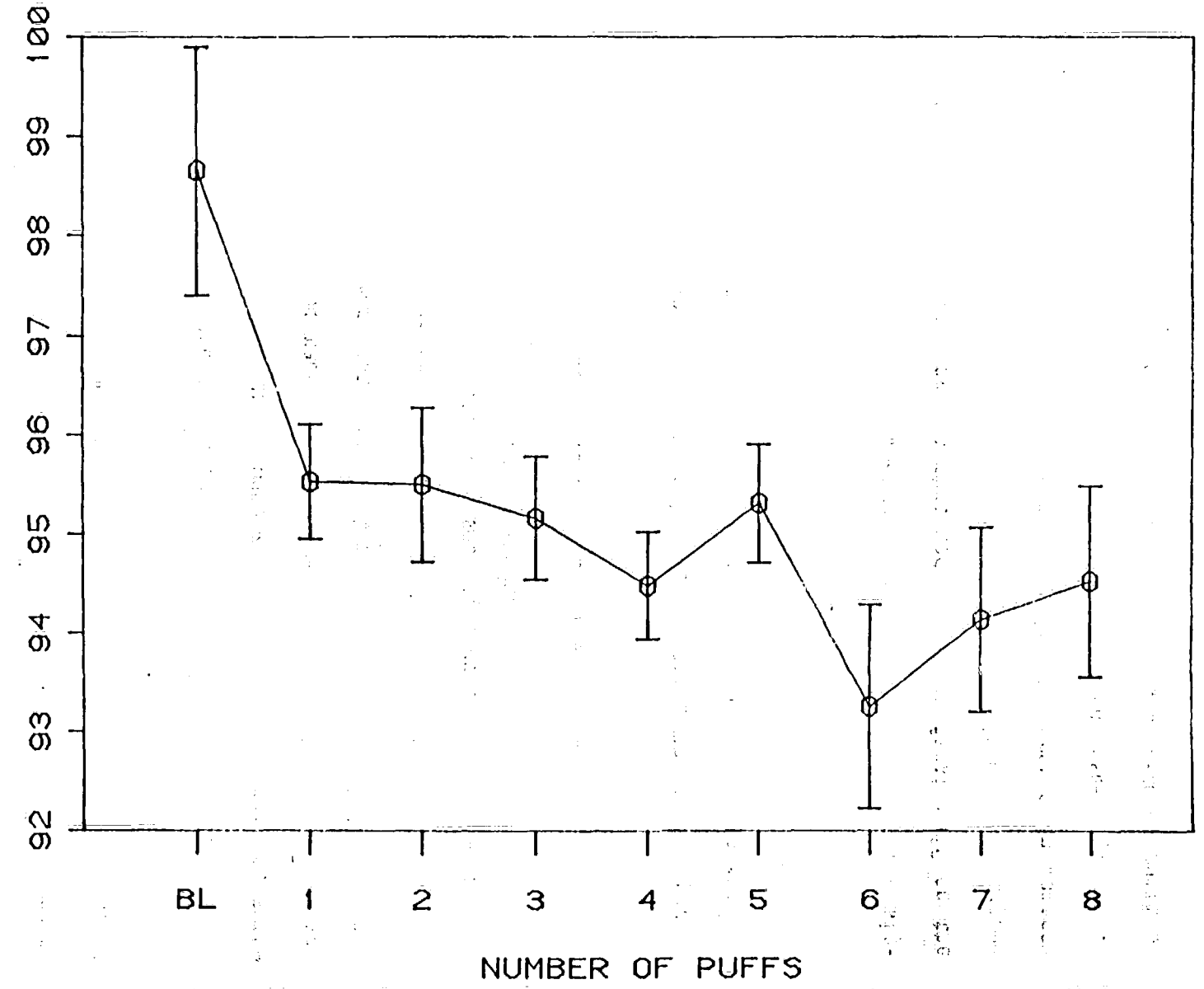
Figure 5.

PUFF X PUFF ANALYSIS - P100 LATENCY - OVERNIGHT DEPRIVATION




Puff by puff analysis of $P_{100}$ latency. Overnight smoke deprivation condition. Vertical lines through data points represent standard error values. (n = 10 PREPs per data point.)

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nicotine in mainstream smoke) cigarette, when measurements are made after the entire cigarette is smoked.

Another interesting observation was that the maximal $P_{100}$ latency decrease from pre-smoking baseline values was obtained on the sixth puff of the cigarette (see Figure 5). The magnitude of this effect was 5.39 msec, which is greater than any average latency shift thus far observed, when measurements have been made after the entire cigarette was smoked.[2,3] These data suggest that when measurements are made subsequent to smoking, only the trailing end of the phenomenon is being recorded; the effect has already begun to dissipate. Similar effects have also been observed in experiments on cigarette smoking and heart rate.[8,9]

PREPs recorded when the subjects were not smoke deprived, did not show a statistically significant decrease in $P_{100}$ latency until the fourth puff (see Figure 6). Puffs 5, 6 and 8 were also statistically different from pre-smoking baseline values. The latency effect on puff 7, however, was not significant. In agreement with the results obtained in the overnight smoke deprivation condition, the maximal latency shift was obtained on the sixth puff. The magnitude of this effect was 3.80 msec.

It should be noted that a statistically significant decrease in $P_{100}$ latency was obtained after the very first puff in the overnight smoke deprivation condition, while statistical significance was not obtained until the fourth puff when the subjects were not deprived. A partial explanation for this finding is suggested by an examination of the pre-smoking baseline values in the two conditions. When the subjects were overnight smoke deprived, the pre-smoking baseline latency of the $P_{100}$ was 98.65 msec. When the subjects were not deprived, the $P_{100}$ latency value was 97.06 msec. These data suggest

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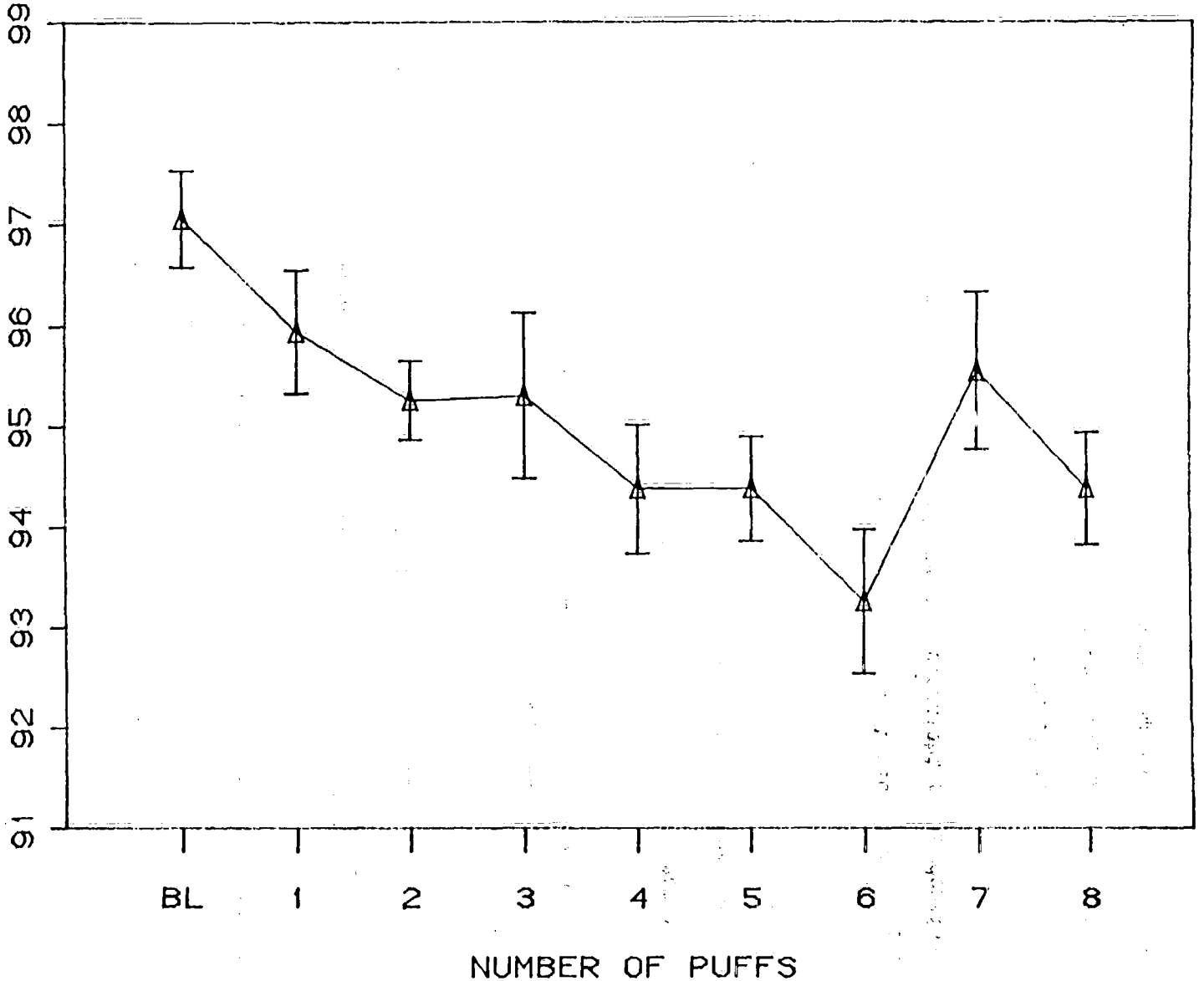
Figure 6.

PUFF X PUFF ANALYSIS - P100 LATENCY - NO DEPRIVATION




Puff by puff analysis of $P_{100}$ latency. No deprivation condition. Vertical lines through data points represent standard error values. (n = 10 PREPs per data point.)

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that in the non-deprived condition, the subjects were still experiencing the residual CNS effects of the previous cigarette.

The results of this experiment are interesting in several respects. First, when smoking machine nicotine analyses are performed, the data reveal that the first three puffs release an average of 10% of the total available nicotine per puff.[10] For example, in the machine smoking of a cigarette that delivers 1.0 mg nicotine in mainstream smoke, 0.1 mg nicotine is collected on the Cambridge pad on each of the first three puffs.[10] This would suggest that the subjects in the current study were obtaining approximately 0.1 mg nicotine on the first puff. Since in the overnight smoke deprivation condition, a statistically significant reduction in $P_{100}$ latency was obtained after the first puff, the data suggest that only an extremely small amount of nicotine is necessary to produce CNS effects. It should be recalled, that the cigarettes used in this experiment were treated with nicotine citrate. It is possible, therefore, that because of uneven treating or other factors, more nicotine was released on the first puff than would happen with naturally occurring nicotine. This explanation seems unlikely, since the data obtained in the non-deprived condition show a gradually increasing nicotine effect. It would seem more likely to postulate that the results reflect a type of CNS super-sensitivity brought about by abstinence.

Second, the data indicate that maximal CNS effects of smoking are obtained prior to the last puff (the sixth of eight puffs in this study). These results were obtained in both deprived and non-deprived conditions. At present, no reasonable explanation for this finding has been determined. It is possible, however, that the subjects did not precisely follow smoking instructions,[3] and that because of harshness, impact or other factors, they did not puff as vigorously on the last two puffs as they did on the earlier puffs.



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Finally, the data indicate that the CNS effects of smoking, as measured by the PREP, are greater than previously demonstrated.[2,3] In actuality, the effects are greatest during smoking, and they begin to dissipate by the time the cigarette is extinguished. Post-smoking studies on the PREP, therefore, may be measuring an effect which has diminished by as much as 50%.

E. Dose/Response Study (In Progress)

In 1982, results of experiments comparing PREP data obtained on experimental cigarettes with those obtained on market-brand cigarettes were reported.[2] Employing a dose/response curve relating nicotine delivery to $P_{100}$ latency shifts, it was demonstrated that as nicotine delivery was increased $P_{100}$ latencies decreased in a dose-related manner (see Figure 7).

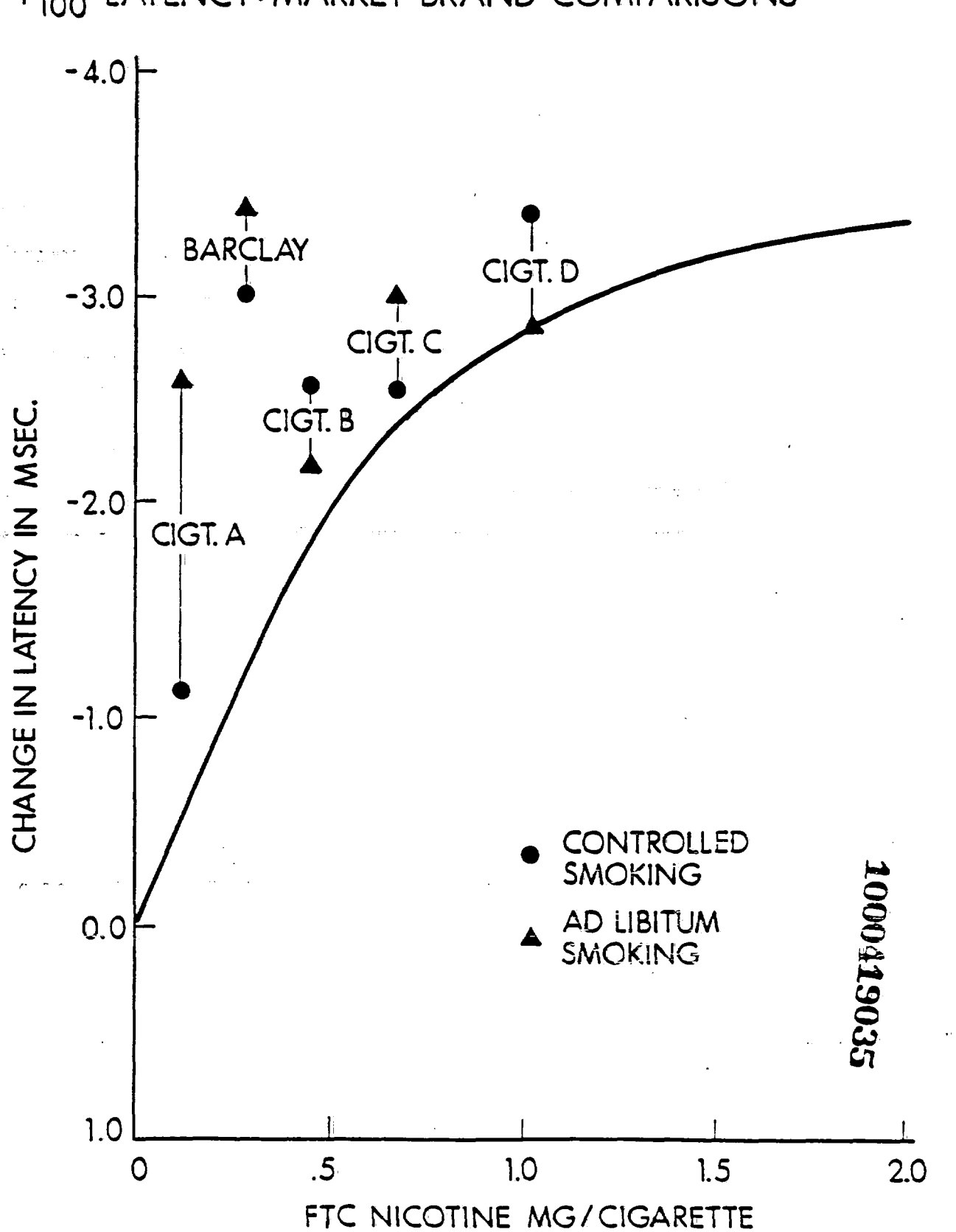
The data which generated the curve in Figure 7 were based upon 28 subjects and 5 experimental cigarettes. In the construction of the curve, data from a number of different studies were employed, where cigarette type, deprivation conditions and other factors were varied. Therefore, the curve represented only an approximation of what could be expected in a study where all relevant variables were controlled within a single experimental paradigm. Nonetheless, it was possible to demonstrate that when subjects smoked market-brand cigarettes in a controlled manner[2] there was remarkably good agreement between what was obtained on the $P_{100}$ latency and what would be predicted, based upon the curve.* In the ad libitum phase of the experiment, similar results were obtained, except in the case of an ultra low nicotine delivery (0.1 mg) cigarette. In this instance, a far greater latency decrease than was

*These conclusions do not apply to Barclay, which produced effects comparable to those obtained with a cigarette delivering 1.0 mg nicotine.



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

Figure 7.




Best-fit curve relating magnitude of $P_{100}$ latency shift to nicotine delivery. The curve is based upon 96 data points from 28 subjects. Market-brands are located on the curve with respect to F.T.C. nicotine deliveries.



Source: https://www.industrydocuments.ucsf.edu/docs/gmml0000

predicted was actually obtained. These findings were interpreted as convincing evidence in support of the nicotine titration hypothesis.

In order to conduct more systematic dose/response studies, it would be necessary to control all relevant variables within a single experiment. Toward this aim, the study described below was initiated.

Thirty R&D employees are serving as subjects in the study. All relevant variables, including smoke deprivation, are under experimentor control. All the cigarettes that are being used in the study were made from the same batch of filler which was a pre-1962 Marlboro blend (see Table 2). The filler contains only strip tobacco and standard flavors, except after-cut. The entire batch of filler was denicotinized by steam/ammonia treatment.[11] Then nicotine citrate, in a vehicle of 95% ethanol, was sprayed on all cigarettes except CLI, to achieve the different nicotine delivery levels. Only vehicle was sprayed on cigarette CLI.

Table 2

ANALYTICAL DATA FOR CIGARETTES BEING USED IN THE DOSE/RESPONSE STUDY

| | CLI | CLJ | CLK | CLL | CLM | CLN | CLO | CLP |
|---|---|---|---|---|---|---|---|---|
| Nic., mg/cigt. | 0.17 | 0.40 | 0.72 | 0.93 | 1.14 | 1.31 | 1.52 | 1.65 |
| Tar, mg/cigt. | 12.1 | 12.3 | 12.9 | 13.9 | 13.8 | 15.0 | 14.6 | 15.0 |
| RTD, in $H_2O$ | 3.4 | 3.3 | 3.3 | 3.4 | 3.4 | 3.3 | 3.3 | 3.3 |
| CO, mg | 8.3 | 8.4 | 8.6 | 9.4 | 8.8 | 9.1 | 9.8 | 9.4 |
| NO, mg | 0.17 | 0.18 | 0.18 | 0.18 | 0.18 | 0.19 | 0.19 | 0.18 |
| Puff Count | 10.5 | 10.6 | 10.7 | 10.2 | 10.4 | 10.3 | 10.6 | 10.7 |
| Dilution (%) | 43.0 | 43.4 | 42.8 | 43.2 | 41.7 | 41.2 | 41.8 | 42.1 |

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Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

As Table 2 indicates, the cigarettes are relatively invariant in smoke constituents other than nicotine. The nicotine deliveries range between 0.17 and 1.65 mg/cigarette with a mean difference among cigarettes of 0.23 mg (SD = .07 mg).

In addition to the smoking of the eight experimental cigarettes, two control conditions have been included in the study. One control consists of the sham smoking of an unlit cigarette. A second control consists of smoking a "zero nicotine delivery" cigarette (80 mm Cambridge). It is expected that these two conditions will be identical in terms of having no effect on $P_{100}$ latency.

The experiment should be completed by the end of 1983. The first step upon completion, will be to construct a new curve relating nicotine delivery to PREP $P_{100}$ latency effects. At that time, it will be possible to conduct a number of important studies. For example, it was previously demonstrated that under ad libitum smoking conditions, it was possible to obtain CNS effects with low delivery cigarettes which were comparable to those obtained with high delivery cigarettes.[2]

A question arose as to the applicability of these findings to particular classes of smokers. That is, in the earlier study, the average nicotine delivery of the brands the subjects normally smoked was approximately 0.7 mg/cigarette. Would similar results be obtained with subjects where the nominal nicotine deliveries of their usual cigarettes differed from 0.7 mg? For example, would ultra low delivery smokers also titrate low delivery cigarettes? It will be possible to obtain data relevant to this question by testing the ultra low delivery subset of subjects in the current study. A similar question could be asked with respect to high delivery smokers (see Plans).

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Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

In conclusion, the PREP is a fast, reliable and sensitive technique for assessing CNS responses to cigarette smoking. Upon completion of the study described above, it will be possible to address many of the lingering questions on the relationship between smoking behavior and the brain.

## F. Conclusions (PREP Studies)

### 1. General Considerations

a. The PREP is a reliable technique for assessing the CNS effects of smoking.

b. PREP $P_{100}$ latency effects are attributable, at least in part, to nicotine.

### 2. Return to Baseline Study

a. The effects of smoking on PREP $P_{100}$ latencies are maximal immediately post-smoking.

b. $P_{100}$ latencies return to their pre-smoking baselines between 50 and 60 minutes post-smoking.

### 3. Smoking Over A Four Hour Period

a. $P_{100}$ latencies tend to approach pre-smoking baselines just prior to when the subjects smoke their next cigarette.

b. PREP $P_{100}$ latencies are maintained below pre-smoking baselines when subjects are allowed free access to cigarettes.

### 4. Puff-by-Puff Analysis of Cigarette Smoking

a. The data suggest that only an extremely small amount of nicotine (i.e., approximately 0.1 mg, delivered in mainstream smoke) is necessary to produce CNS effects.

b. CNS effects are greater during smoking than immediately post-smoking.

### 5. Dose/Response Study

a. Dose/response relationships between nicotine and $P_{100}$ latency are being established within a single experimental paradigm.

b. It will be possible to systematically study the degree to which titration occurs among various classes of smokers.

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Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

## V. EVOKED POTENTIALS TO OLFACTORY AND TRIGEMINAL STIMULANTS (EPs)*

### A. General Considerations

Odor quality evaluation is an area of research which is of critical importance to Philip Morris. In a positive sense, odor quality evaluation is relied upon in order to determine desirable smoke properties. Similarly, these evaluations are utilized in eliminating undesirable properties such as ash tray odors and irritative factors in sidestream smoke.

Until very recently, Philip Morris has had to rely almost exclusively upon the subjective reports of panelists for product evaluation. Subjective responses are difficult to evaluate since the internal criteria (e.g., response biases) that generate the responses are not readily determinable.

It would be extremely useful if there were an odor quality evaluation technique that was objective, non-verbal and quantifiable using standard parametric statistical techniques. EP research may be at least a partial answer to the problem of objective odor quality evaluation.

EP research has a history which dates back approximately seventeen years, with the pioneering work of Finkenzeller[12] and Allison and Goff.[13] These researchers appeared to be successful in obtaining EPs from the human scalp. However, it was later demonstrated[14] that these responses were not true EPs, but were most likely artifacts associated with the manner in which the stimuli were presented.

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*In the literature, these responses have been termed olfactory evoked potentials (OEPs). However, the cortically recorded response to stimulation of the sense of smell may contain both olfactory and trigeminal components. Therefore, to avoid confusion, evoked potentials to olfactory and trigeminal stimulants will be referred to solely as EPs.



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

Subsequent progress in the area of EP research languished until the difficulties associated with stimulus presentation were overcome. This breakthrough occurred in the late seventies when K-H. Plattig and G. Kobal[15,16] developed a technique for presenting stimuli in a manner suitable for recording artifact-free potentials.

The technique developed by Plattig and Kobal allows for the presentation of stimuli within a constantly flowing stream of warm, moist air. The rise time to vapor saturation of the stimulus is 20-30 msec, sufficient for computer signal averaging. The technique also avoids or controls artifact contaminations such as thermal disturbances, auditory responses, somatosensory potentials, etc.

EP studies employing the Plattig and Kobal technique have been in progress at R&D since June, 1982. In addition, the studies employ an olfactometer which was designed and constructed by Dr. Kobal.

The subjects for the EP studies were three female and six male R&D employees. The general procedure for recording the EPs has been described elsewhere.[17] In the studies described below, 15 responses were obtained for each stimulus presentation and averaged off-line. Artifact rejection was accomplished by visual inspection of each waveform prior to averaging. Between one and four averaged EPs were obtained in an experimental session. An example of an EP with the components labeled is illustrated in Figure 8. The same labeling nomenclature applies to all subsequent EPs.

The stimuli that were used in the studies were d-limonene (SCM, Terpenene Products) methyl salicylate (Givaudin, Inc.) and $CO_2$ (Air Products, Inc.). The purity of the limonene and methyl salicylate were determined by capillary gas chromatography.[18] The analysis revealed that the purity of the limonene was 99.6% and that the purity of the methyl salicylate was 99.9%.



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

Figure 8.

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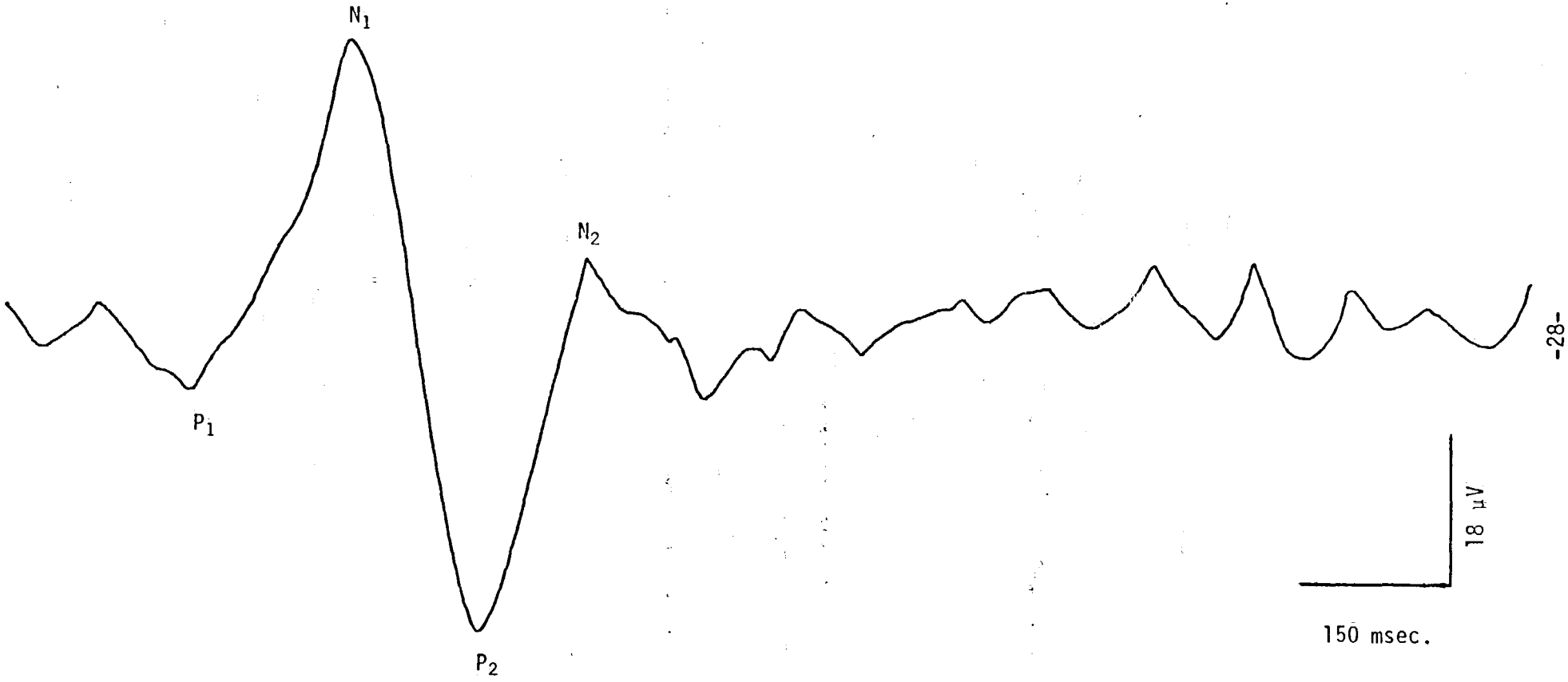




Evoked potential with components labeled. The same labeling nomenclature applies to all subsequent evoked potentials. Negative up.

Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

B. Flow Rate Determinations

This experiment was undertaken in order to determine the optimal flow rates necessary to obtain clear EPs to the stimuli under study. The stimuli were limonene, $CO_2$ and a 50% mixture of limonene and $CO_2$.

Initial studies were conducted using a flow rate of 94 ml/sec. Well-formed EPs were obtained to 100% $CO_2$ and to the limonene + $CO_2$ mixture in all subjects. Clear limonene potentials, however, were obtained in only four of nine subjects (see Figure 9).

When the flow rate was increased to 134 ml/sec, well-formed EPs were obtained to all stimuli (see Figure 9). Even when the vapor saturated stimuli were diluted 50% by the addition of odor-free air, reasonably clear responses were visible.

There are probably two related reasons for the lack of uniform success with limonene at the 94 ml/sec flow rate. First, limonene potentials are, in general, small relative to those obtained with more potent odorants such as isoamyl acetate.[16] Given this fact, it is possible that the limonene potentials were obscured by artifacts derived from recordings on relatively unpracticed subjects (these experiments were carried out early in the program). Second, at low flow rates olfactory stimuli tend to diffuse in the nasal cavity before reaching the receptors in the olfactory mucosa, resulting in a desynchronized discharge of the participating neural elements. The end result of this desynchronization would be smaller potentials.

These studies indicate that flow rate is an important variable in EP recordings. For weak olfactory stimuli, it is necessary to use a higher flow rate than for more potent stimuli. The remaining experiments reported here employ a flow rate of 134 ml/sec.

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Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

Figure 9.

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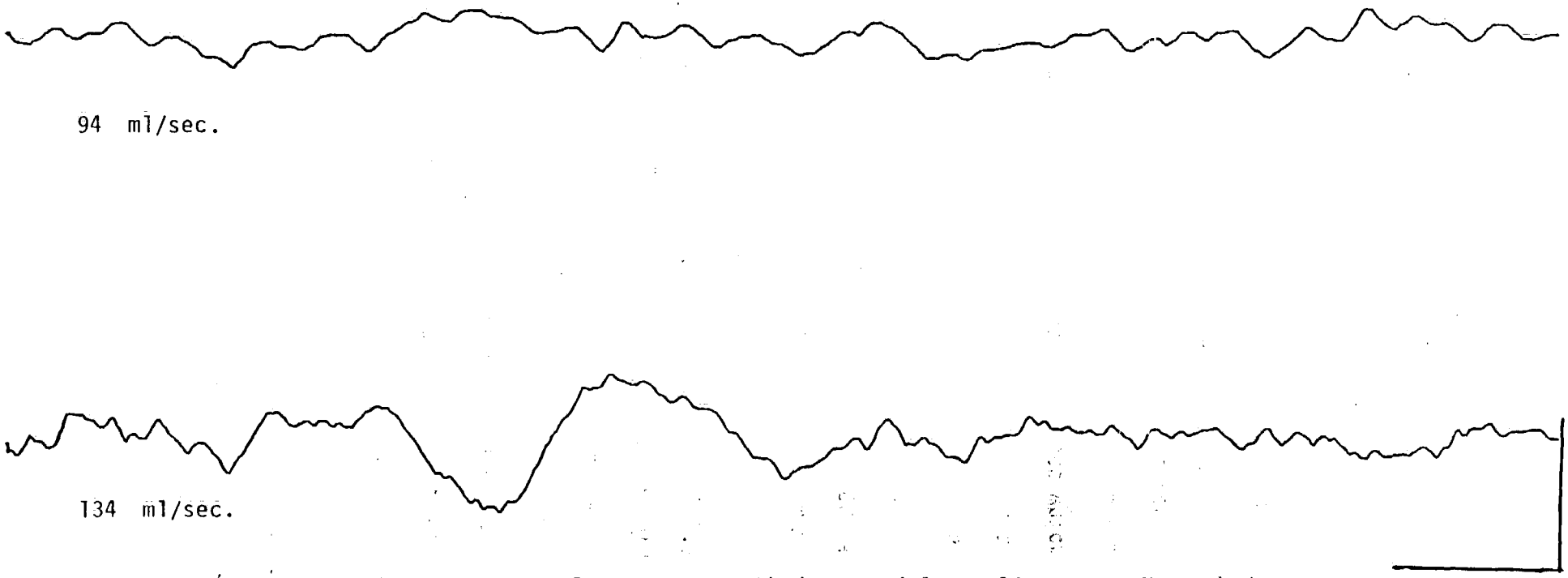
EVOKED POTENTIALS TO LIMONENE AT TWO FLOW RATES




The effects of flow rate on evoked potentials to limonene. No evoked potential is discernible at the 94 ml/sec. flow rate.



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

C. EPs to Limonene, Methyl Salicylate, $CO_2$ and Mixtures

Do different chemical stimuli produce EPs that are different? If so, it might be possible to characterize the stimuli in terms of the effects they produce on EP latency, amplitude and/or waveform features. Investigators would then be in a position to obtain objective data on the odor quality of tobacco flavorants and smoke constituents.

An area of research where objective quantification would be particularly useful is in the study of feeling factors. These include the sensations of pungency, spiciness, cooling, warmth and irritation, and are thought to be mediated by the trigeminal nerve.[19,20] Since the response of the trigeminal nerve is believed to be represented in the cortically recorded EP,[15,16] it should be possible to obtain numerical data on feeling factors. This would be a valuable contribution, since feeling factors have been postulated to be important determinants of cigarette smoke flavor.[21]

The stimuli which were used in the study included limonene, methyl salicylate and $CO_2$. The stimuli differ in several respects. $CO_2$ is an odorless compound, but exhibits feeling factors. The cortically recorded response to $CO_2$ should therefore be purely of trigeminal origin. Limonene is a compound with a citrus, lemon-like odor. At low concentrations it appears to show only odor properties. At high concentrations it produces sensations which are quite similar to those elicited by $CO_2$. Methyl salicylate at low concentrations, also appears to cause only an odor sensation. When concentrations are increased, however, it generates feeling factors which are, in many respects, comparable to those produced by $CO_2$ and high concentrations of limonene.

In this study, a 50% concentration of $CO_2$ was employed. Similarly, limonene and methyl salicylate were tested at 50% vapor saturation. These concentrations should yield trigeminal potentials to $CO_2$ and odor potentials to

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limonene and methyl salicylate.* Therefore, it should be possible to determine: 1) whether the stimuli differ in terms of the EPs they produce; and 2) the difference between odor potentials and trigeminal potentials.

EPs were also obtained to 50% mixtures of $CO_2$ and the two odorants (combination potentials). The goal of the mixture study was to determine: 1) how the addition of an odorant to a trigeminal stimulant affects the EP; and 2) whether elements of both compounds are detectable in the cortically recorded response.

The results obtained for limonene, $CO_2$ and the limonene + $CO_2$ mixture are illustrated in Figures 10 and 11. The methyl salicylate and methyl salicylate + $CO_2$ mixture data are as yet, incomplete and will be discussed separately.

EP latencies for limonene, $CO_2$ and the limonene + $CO_2$ mixture are shown in Figure 10. The latency values for limonene and $CO_2$ were quite similar for each of the components. EPs to the mixture (limonene + $CO_2$), however, were of shorter latencies than to either of the other stimuli.

Amplitude data are presented in Figure 11. Limonene produced the lowest amplitude response on each of the EP components. Larger responses were obtained to $CO_2$. The largest responses, however, were obtained to the limonene + $CO_2$ mixture.

These data suggest that the three stimuli are differentiable in terms of latency and amplitude characteristics. Therefore, at least for these compounds, it is possible to distinguish differences among an odorant, a trigeminal stimulant and a combination of the two.

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*Subjects report no feeling factors to limonene and methyl salicylate at these concentrations.

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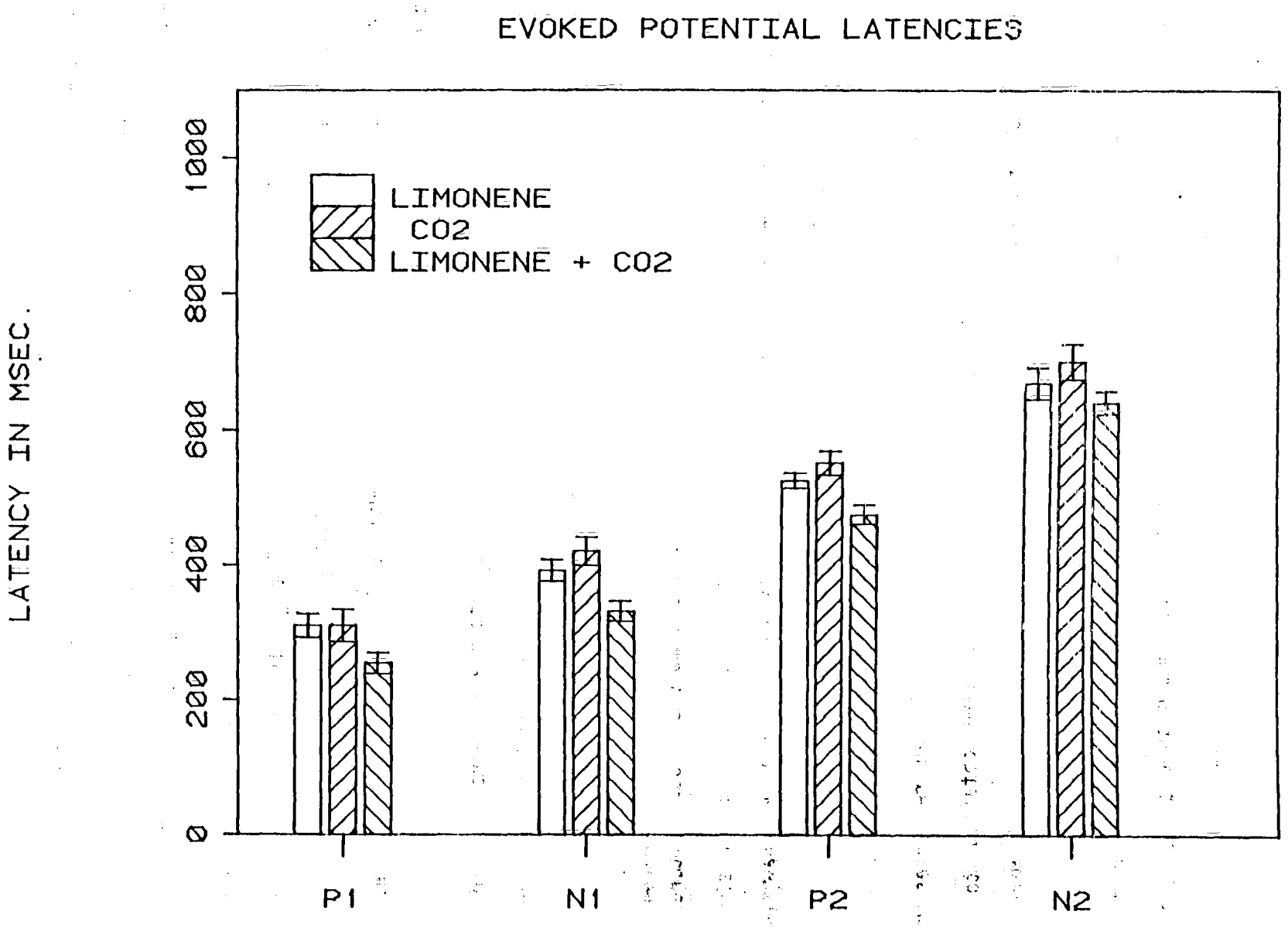
Figure 10.




Evoked potential latencies for limonene, $CO_2$ and the combination of limonene and $CO_2$. Limonene was tested at 50% vapor saturation. $CO_2$ was tested at a 50% concentration. The combination was tested at the same concentrations as above. Vertical lines through bars represent standard errors. (n = 9 EPs per data point for $CO_2$ and limonene, and 18 EPs per data point for the combination.)

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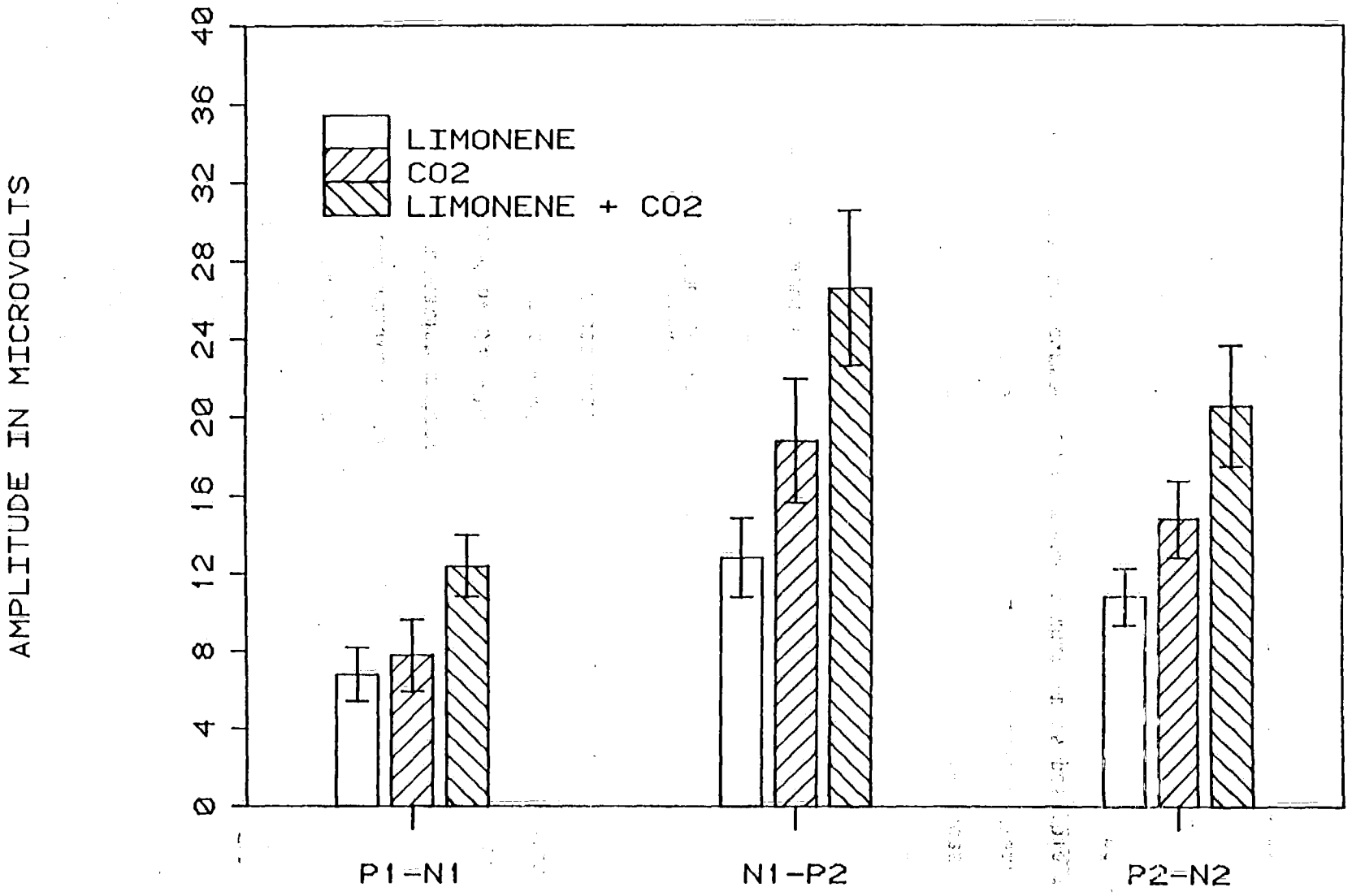
Figure 11.

EVOKED POTENTIAL AMPLITUDES




Evoked potential amplitudes for limonene, $CO_2$ and the combination of limonene and $CO_2$. Limonene was tested at 50% vapor saturation. $CO_2$ was tested at a 50% concentration. The combination was tested at the same concentrations as above. Vertical lines through bars represent standard errors. (n = 9 EPs per data point for $CO_2$ and limonene, and 18 EPs per data point for the combination.)

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Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

In terms of waveform morphology, the limonene + $CO_2$ combination appears to contain elements of both compounds. These findings suggest that it should be possible to use computer techniques for isolating trigeminal (feeling factor) contributions to mixtures. An example of this procedure is illustrated in Figure 12. The EP at the top of Figure 12 is to a mixture of limonene + $CO_2$. The second and third are to limonene and $CO_2$, respectively. The EP labelled Comb. - limonene resulted from a computer subtraction of the limonene potential from the combination potential. The resulting potential is quite similar to that produced by $CO_2$ alone. These findings, although preliminary, suggest the possibility of employing computer techniques for isolating and quantifying feeling factors associated with whole smoke and other complex mixtures of compounds.

Preliminary data on four subjects have been obtained to methyl salicylate and the methyl salicylate + $CO_2$ combination. It appears that methyl salicylate differs from both limonene and $CO_2$ in terms of latency and amplitude characteristics. Latencies for methyl salicylate are shorter than to limonene and $CO_2$. Methyl salicylate amplitudes are greater than those due to limonene, but less than to $CO_2$. The methyl salicylate + $CO_2$ combination produces shorter latency, higher amplitude responses than the limonene + $CO_2$ combination.

The results of the current study suggest that it is possible to distinguish among stimuli in terms of their waveform characteristics,* and that it should be possible to quantify feeling factors in the cortically recorded potential. A great deal of additional research is necessary before the technique becomes useful in product evaluation, but preliminary evidence is extremely encouraging.

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*Data from G. Kobals laboratory (see pp 45-48) lend additional support to these conclusions.



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Figure 12.

EVOKED POTENTIALS

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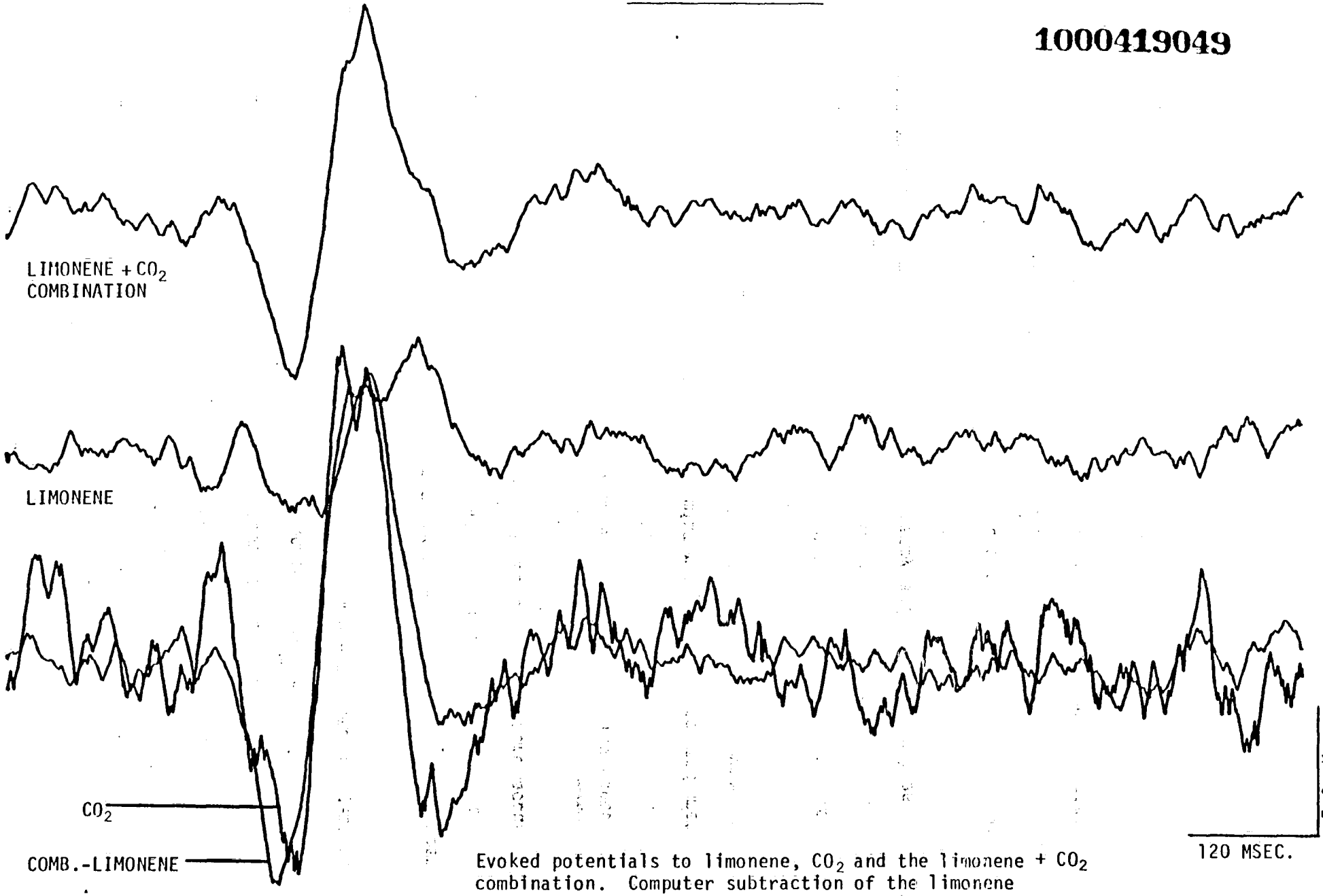


Evoked potentials to limonene, $CO_2$ and the limonene + $CO_2$ combination. Computer subtraction of the limonene potential from the combination reproduces the $CO_2$ potential (see text).



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

D. Concentration/Response Studies (In Progress)

In the previous experiment, data were presented which indicated that it was possible to differentiate among olfactory and trigeminal stimulants in terms of EP waveform characteristics. This study addresses the issue of whether it is possible to obtain information about stimulus intensity from the EP. That is, do EPs change in a systematic manner in response to changes in concentration? If this were the case, it would be possible to obtain objective data on human responses to stimulus intensity.

A second purpose of this study is to examine the relationship between psychophysical estimates of stimulus intensity and EPs. The question of interest is to determine which of the two measures provides a more accurate index of physical stimulus intensity (i.e., concentration).

In order to answer these questions, experiments are being conducted on EPs to three concentrations of limonene, methyl salicylate and $CO_2$. In addition, studies are being performed on mixtures of limonene and methyl salicylate with 25, 50 and 75% $CO_2$. Concurrent with EP recording, psychophysical data on perceived stimulus intensity are being obtained.

Preliminary data on three concentrations of limonene, methyl salicylate and $CO_2$ are illustrated in Figures 13 and 14. It is apparent that concentration had systematic effects on EP latency and amplitude values. As concentrations increased, EP latencies tended to decrease (Figure 13), while amplitudes tended to increase (Figure 14). These effects were apparent with all three stimuli, but were particularly dramatic in the case of $CO_2$.

The psychophysical data on perceived odor intensity are illustrated in Figure 15. As with the EPs, there is good general agreement between stimulus concentrations and perceived intensity. As stimulus concentration

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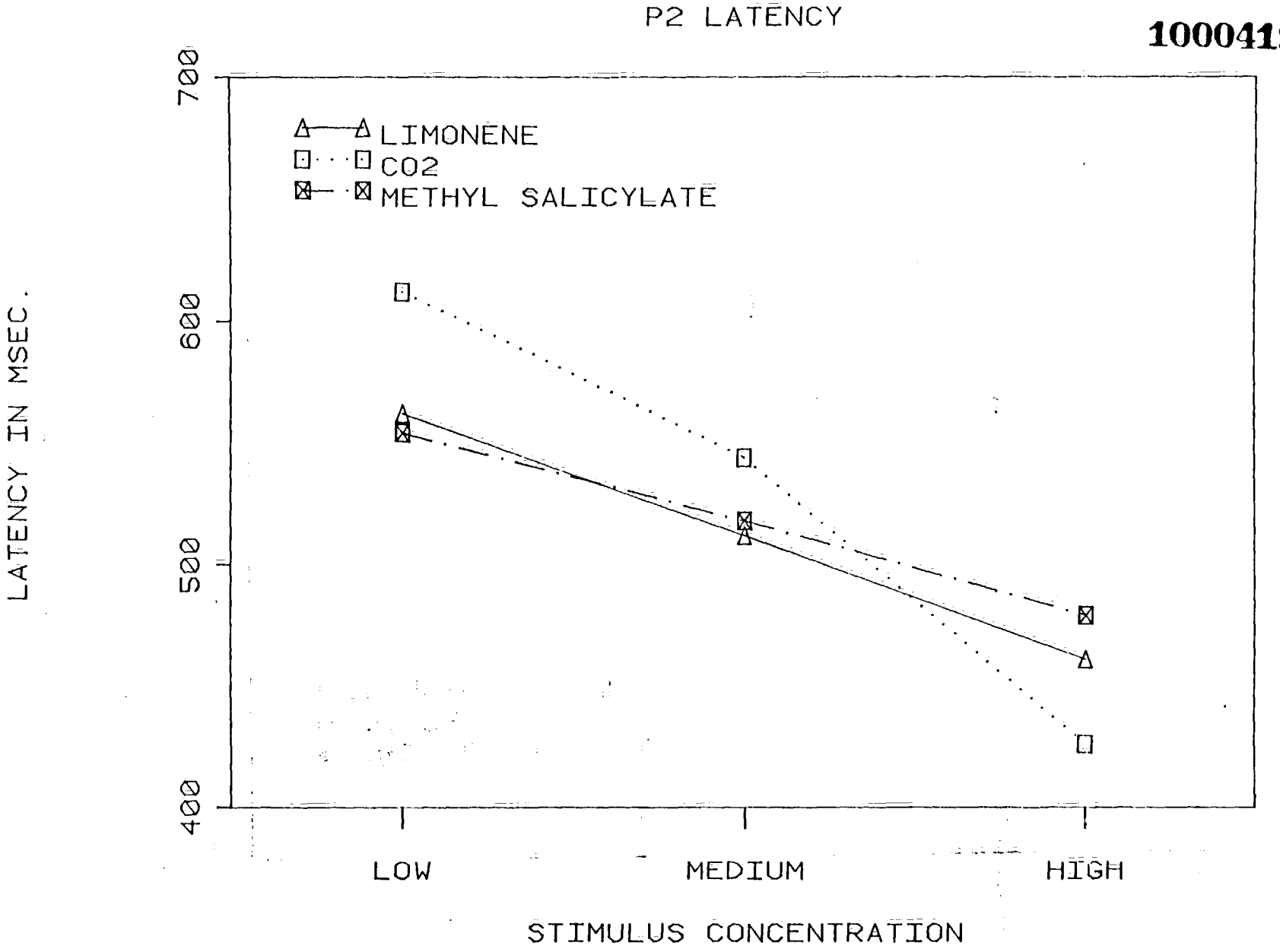
Figure 13.




The effects of stimulus concentration on EP latency. As stimulus concentration increases, EP latencies decrease (n = 5 EPs per data point).

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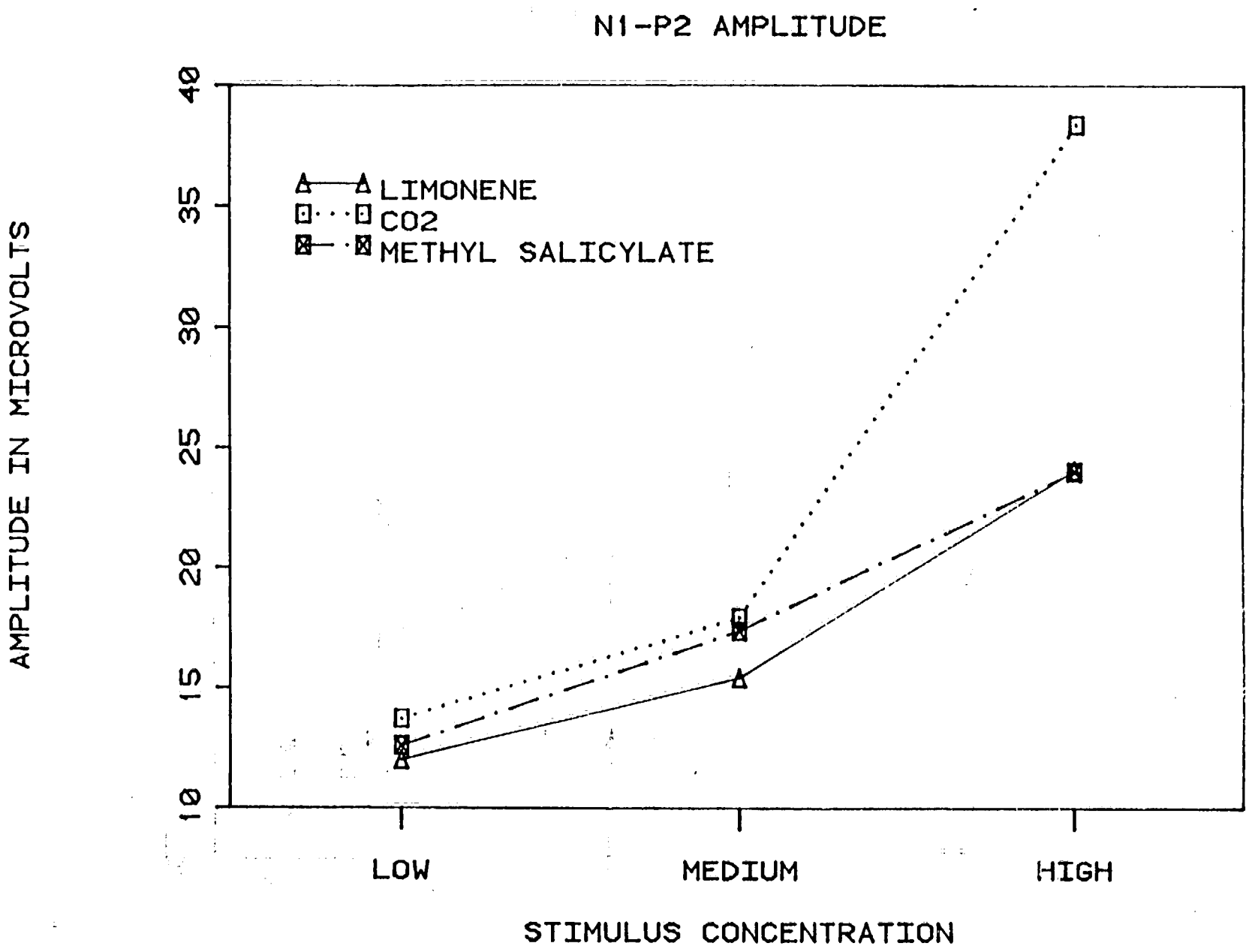
Figure 14.




Effects of stimulus concentration on EP amplitude. As concentrations increase, EP amplitudes increase (n = 5 EPs per data point).



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Figure 15.

PSYCHOPHYSICAL DATA

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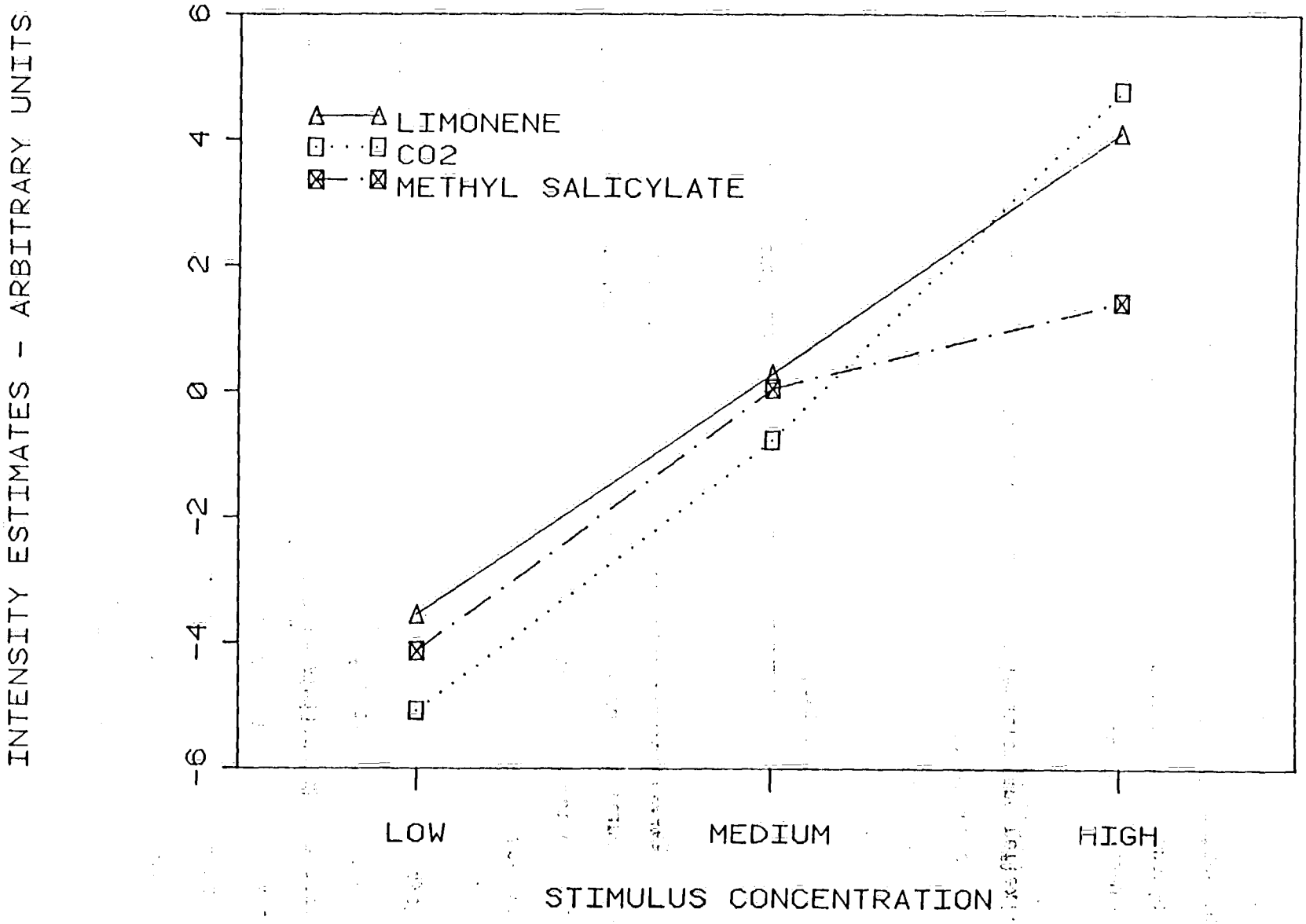


The effects of stimulus concentration on psychophysical estimates of intensity. For limonene and $CO_2$, estimates increase as concentrations increase. For methyl salicylate, there is little difference in the estimates to medium and high concentrations (n = 5 sets of 15 estimates per data point.)



Source: https://www.industrydocuments.ucsf.edu/docs/gmnl0000

increased, the psychophysical judgments of intensity increased. An exception are the judgments relative to medium and high concentrations of methyl salicylate, where the subjects appeared to detect little difference between the two. This finding is particularly interesting in light of the fact that methyl salicylate EP latency and amplitude values for medium and high concentrations were clearly differentiable. The data suggest the possibility that a rather precise measure of stimulus concentration is made by the brain, but some of this precision is lost during the interpretive process involved in psychophysical judgment.

Although preliminary, the results of the current study are very encouraging. It appears that EPs can provide objective information about stimulus intensity. In fact, the data suggest that in some instances, EPs may be superior to psychophysical judgments in estimating concentrations.

## E. Electrotrigeminograms (ETGs): Preliminary Studies

The trigeminal nerve is widely distributed throughout the head. Branches of the nerve are found in the mouth, cornea and nose.[22] Stimuli which activate the trigeminal nerve are often classified as noxious or irritating in nature.[19,20] Consequently, stimulation of this nerve is often associated with physical defensive reactions such as lacrymation, salivation and reflexive inhibition of respiration.[19]

It is a uniquely human phenomenon that certain degrees of trigeminal stimulation are tolerated, and even become associated with pleasure. Capsaicin in chili peppers, $CO_2$ in carbonated beverages, menthol in candy, cigarettes and medicines, all are desirable in controlled amounts. These preferences are learned, since trigeminal stimulants are uniformly noxious to neonates.[20]

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It has been postulated[21] that the impact associated with cigarette smoking is, at least in part, due to stimulation of the nasal and oral branches of the trigeminal nerve. Specifically, it has been presumed that feeling factors are produced by trigeminal stimulation. Probable stimulants in tobacco smoke include nicotine, $CO_2$, ammonia, numerous tobacco flavorants such as menthol, and other smoke constituents.

In order to determine which smoke constituents and tobacco flavorants are trigeminal stimulants, and to subsequently quantify them, it would be desirable to obtain responses directly from the trigeminal nerve. Fortunately, such a technique has recently been developed.[23]

G. Kobal has demonstrated that when stimuli are presented at high concentrations, a negative potential can be recorded from the human respiratory mucosa. It is postulated that the negativity reflects the depolarization potential of nasal trigeminal free nerve endings. Kobal is currently in the process of verifying this interpretation in animals by simultaneously recording activity from the respiratory mucosa and trigeminal afferent fibers. Preliminary studies on the response which Kobal has termed the electrotrigeminogram (ETG) have been conducted at R&D. Thus far, ETGs to limonene, methyl salicylate and $CO_2$ have been recorded in two subjects.

Examples of ETGs recorded to 100% $CO_2$ at two different stimulus durations are illustrated in Figure 16. The shorter stimulus duration (200 msec) produced a positive potential. The longer stimulus duration (1000 msec) produced a high amplitude negativity. The significance of the positivity has not been determined, but the negative potential is correlated with the sensation of pain.[23]

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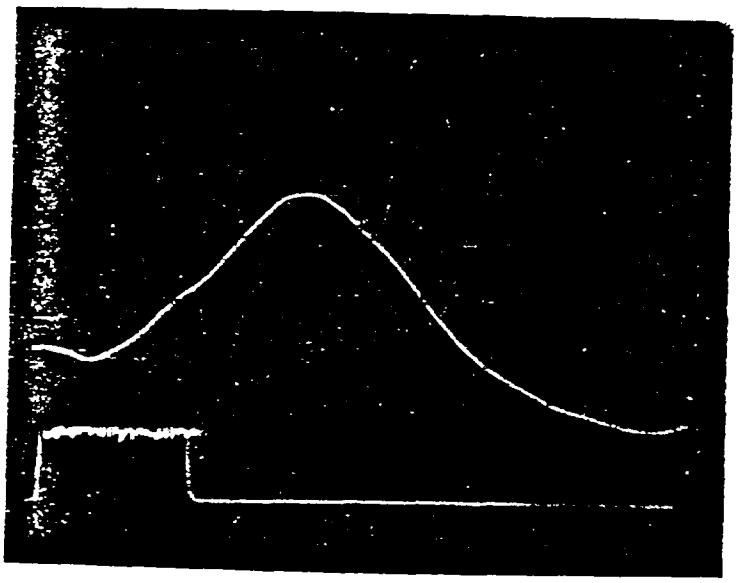
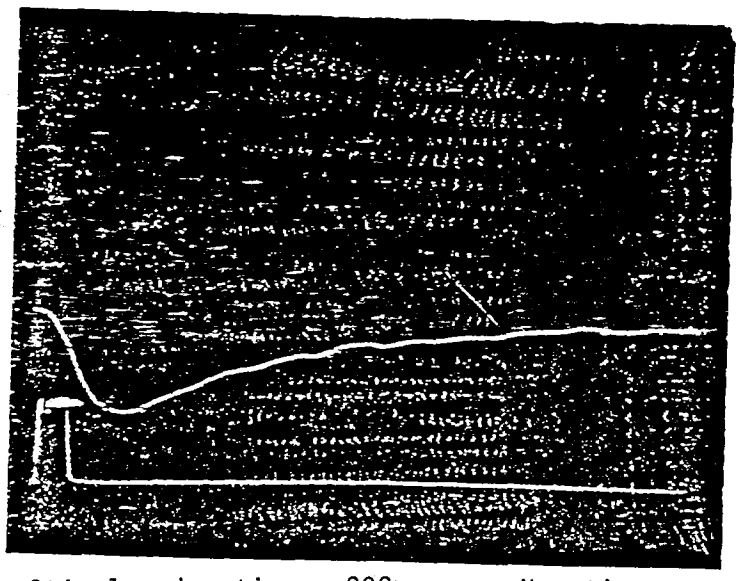
Figure 16.

Electrotrigeminograms to $CO_2$




Stimulus duration = 1000 msec. Negative up.




Stimulus duration = 200 msec. Negative up.

ETGs to 100% $CO_2$ . Stimulus duration = 1000 msec. in upper trace and 200 msec. in lower trace. Negative up. Note that 200 msec. pulse of $CO_2$ produces only positivity. The 1000 msec. pulse produces a large negative potential.

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Similar results were obtained when limonene and methyl salicilate (at vapor saturation) were used as stimuli. That is, longer stimulus durations produced a negative potential and also a sensation of irritation.

At this time, it is not known how feeling factors such as spiciness, pungency, etc., will be represented in the ETG. The stimulus intensities necessary to produce feeling factors might be somewhere between those that produce only odor and those that produce pain. Therefore, one might expect a negative potential, but one of a lesser magnitude than occurs when pain is produced. Alternatively, the ETGs to stimuli interpreted as pleasurable (i.e., feeling factors) and those interpreted as painful might be quite similar. Since pain possesses psychological dimensions, perhaps context would determine how the stimulus is perceived.

It will also be interesting to determine whether different classes of stimuli producing feeling factors also produce morphologically different ETGs. For example, will the ETGs produced by menthol (cooling) be different than those to cinnamaldehyde (pungent)? Results from Kobal's laboratory[23] suggest that this may be the case. He has demonstrated, for example, that the ETGs to isoamyl acetate, eucalyptol, linalool and $CO_2$ are different in waveform characteristics.

Work continues, with the goal of developing ETG recordings into a readily usable technique. The procedure is somewhat difficult, and it requires the use of highly trained and cooperative subjects. In the near future it is planned to begin testing two additional subjects and to continue to develop the technique employing limonene, methyl sallicylate, $CO_2$ and combinations of these three stimuli. Plans also include simultaneous recordings of both ETGs and EPs.

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F. EPs - G. Kobal, Erlangen, W. Germany

In March, 1983, Gerd Kobal presented the preliminary results of EP experiments which were partially funded by Philip Morris. The studies were performed on 13 subjects using menthol, vanillin, $CO_2$ and the combination of vanillin + $CO_2$ as stimuli.

The latency data are summarized in Figure 17. Shortest latencies were obtained to the vanillin + $CO_2$ combination. Vanillin alone produced the longest latencies, whereas $CO_2$ alone produced latencies somewhat longer than the combination. Menthol produced latencies which were intermediate between $CO_2$ and vanillin.

The amplitude data are illustrated in Figure 18. As the figure indicates, the amplitude data for the four stimuli exactly compliment those obtained on latency. The combination produced the largest EPs, while vanillin produced the smallest potentials. $CO_2$ potentials were not quite as large as those produced by the combination, while menthol EPs were intermediate to those obtained with vanillin and $CO_2$.

The psychophysical data from these studies indicate that the vanillin + $CO_2$ combination was rated higher in overall intensity than any of the other stimuli. $CO_2$ was rated as second in intensity, and menthol was third. Vanillin was rated as least intense.

Kobal's findings are in essential agreement with the data obtained for limonene, $CO_2$ and the limonene + $CO_2$ combination in R&D experiments. It will be recalled that the limonene + $CO_2$ combination produced the shortest latency, highest amplitude responses. $CO_2$ and limonene amplitude data are also in general agreement. However, in the R&D studies it was found that limonene and $CO_2$ latencies were quite similar. The differences between the results of the two laboratories are probably due to the fact that Kobal employed higher

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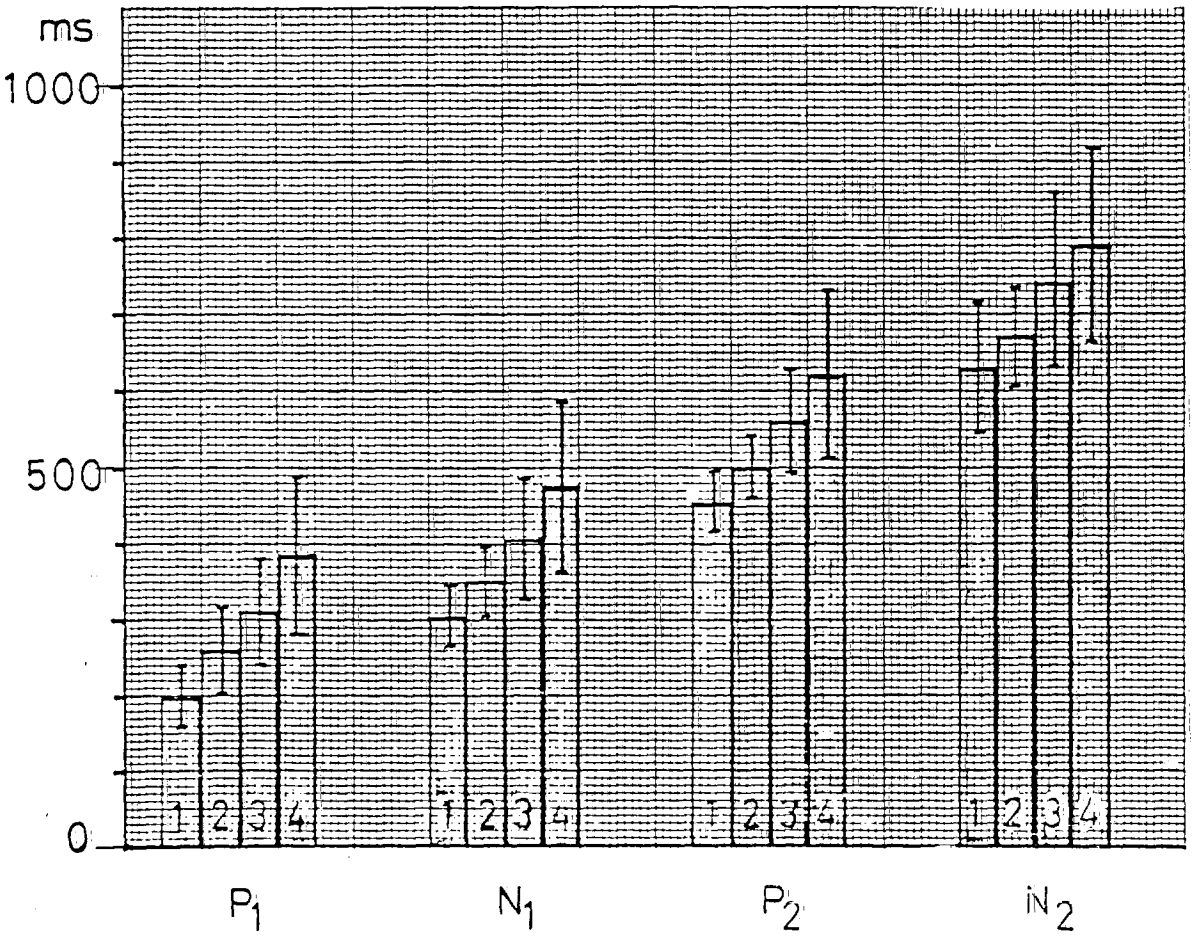
Figure 17.

LATENCIES (MEANS, S.DEV.)




1 = $CO_2$ + Vanillin

2 = $CO_2$

3 = Menthol

4 = Vanillin

Latency data for four stimuli. Components are labeled sequentially. Vertical lines through bars represent standard deviations (n = 26 EPs per data point).

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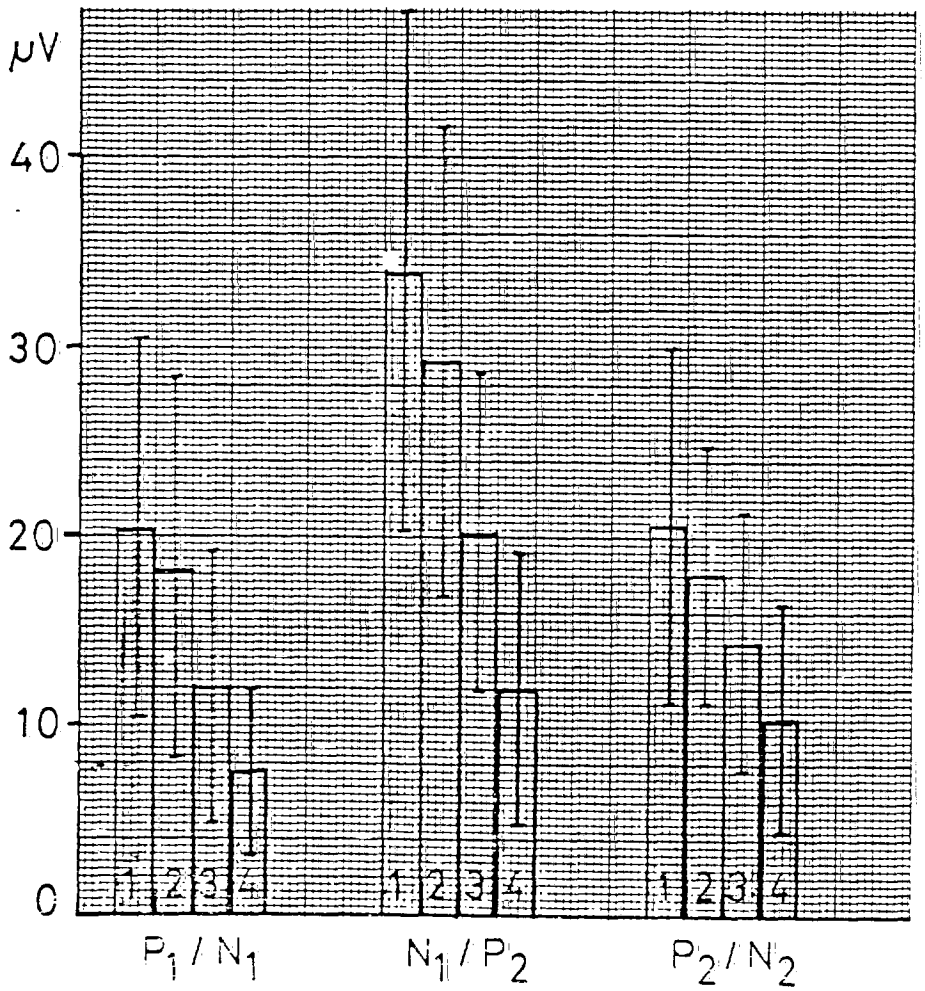
Figure 18.

AMPLITUDES (MEANS, S. DEV.)




1 = $CO_2$ + Vanillin

2 = $CO_2$

3 = Menthol

4 = Vanillin

EP amplitude data for four stimuli. Components are labeled sequentially. Vertical lines through bars represent standard deviations (n = 26 EPs per data point).

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$CO_2$ concentrations. If higher $CO_2$ concentrations had been used in the R&D studies, there would be complete agreement between the findings of the two laboratories.

G. Conclusions: Evoked Potentials to Olfactory and Trigeminal Stimulants (EPs)

1. General Considerations

a. The EP technique is a potential means for obtaining objective, nonverbal data regarding olfactory and trigeminal stimulants.

b. Using EPs, it should be possible to obtain valuable information regarding desirable smoke properties, as well as undesirable properties.

2. Flow Rate Determinations

a. The flow rate of stimulus-laden air is an important factor in EP recordings.

b. Higher flow rates (over 100 ml/sec.) are necessary for weak olfactory stimuli.

3. EPs to Limonene, Methyl Salicylate, $CO_2$, and Mixtures

a. It is possible to distinguish among chemical stimuli in terms of their waveform characteristics (i.e., latency, amplitude and waveform morphology).

b. EPs can provide quantifiable data on important flavor characteristics of cigarette smoke such as feeling factors.

4. Concentration/Response Studies

a. EPs change in a systematic manner in response to changes in stimulus concentration.

b. EPs may be more accurate than psychophysical judgments in estimating concentration.

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5. Electrotrigeminograms (ETGs): Preliminary Studies

a. ETGs should provide a technique for determining and quantifying smoke constituents and tobacco flavorants that are trigeminal stimulants.

b. Different classes of chemical stimuli that produce feeling factors may produce morphologically different ETGs.

6. EPs - G. Kobal, Erlangen, W. Germany

a. The data from Dr. Kobal's laboratory are in agreement with data from R&D.

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## VI. PLANS

### PATTERN REVERSAL EVOKED POTENTIALS (PREPs)

#### 1. Dose/Response Study

It is planned to complete testing of the 30 subjects in 10 smoking conditions by the end of 1983.

#### 2. Titration Study

Subsets of subjects from the Dose/Response Study will be tested to further determine the degree to which titration occurs. It has already been demonstrated that, in a single smoking session, subjects will smoke ultra low delivery cigarettes in such a manner as to obtain CNS effects comparable to high delivery cigarettes (upward titration). It may now be possible to determine:

a. whether upward titration will occur in those subjects accustomed to smoking low delivery cigarettes;

b. whether downward titration will occur in those subjects accustomed to smoking high delivery cigarettes;

c. if upward and downward titration occurs, can the effects be reversed by reversing the normal delivery levels of the cigarettes that the subjects smoke (i.e., high delivery smokers to low and vice versa) for a period of time prior to testing?

### EVOKED POTENTIALS TO OLFACTORY AND TRIGEMINAL STIMULANTS (EPs)

#### 1. Concentration/Response Studies

The plans are to complete the testing of methyl salicylate, limonene, $CO_2$ and $CO_2$ + limonene and $CO_2$ + methyl salicylate combinations at several different concentrations. Eight subjects are currently being tested. It is planned to train and begin testing two additional volunteers.

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2. Flavorant Studies

In collaboration with the Flavor Development Division, additional flavorants will be decided upon and tested. The goal of these studies will be to characterize and to quantify the flavorants in terms of the EPs they produce.

3. Smoke Studies

Upon completion of the new olfactometer currently being designed by the Engineering Department, plans are to begin studies on whole smoke and gaseous smoke constituents. The goal of these studies will be to assist in developing cigarettes with desirable smoke properties and to aid in finding methods to eliminate undesirable smoke properties.

ELECTROTRIGEMINOGRAMS (ETGs)

In the coming year, plans are to develop facility in recording ETGs. Once this is accomplished, systematic studies of stimuli which yield different feeling factor sensations (e.g., cooling, pungency, spiciness, etc.) will begin. The goal of these studies is to be able to classify substances in terms of the types and amounts of feeling factors that they produce. The long term goals will be to assist the Flavor Development Division in finding flavorants which produce feeling factors that smokers interpret as pleasurable.

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